Impacts of the invasive tree *Acacia mearnsii* on riparian and instream aquatic environments in the Cape Floristic Region, South Africa

By

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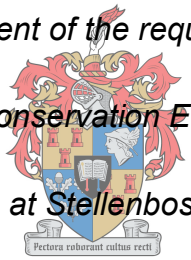

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Declaration

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SUMMARY

In this study, I compared the notoriously invasive wattle species *Acacia mearnsii,* to two native woody species in terms of patterns leaf litterfall and nutrient resorption in riparian environments, and the decomposition of the leaf litter in aquatic environments and in stream macroinvertebrate communities in mountain streams in the Fynbos biome of the CFR. More explicitly, the study assessed: (1) leaf litter fall between *A. mearnsii* and co-occuring native species on an monthly basis (2) the nutrient (N and C) concentrations dropped in leaf litter inputs monthly (3) the amount of nutrients (N and P) resorbed between species before senescence (4) the decomposition rates between *A. mearnsii* and fynbos species in away and home environments to test the Home Field Advantage (HFA) hypothesis and, finally (5) the macroinvertebrate assemblages in different leaf bags in home and away environments to test macroinvertebrate litter affinity effects instream.

The results in the study indicate that *A. mearnsii* had seven to times times higher leaf litterfall rates in the Wit and Du Toit's River compared to co-occuring native species in invaded and near pristine riparian zones. *Acacia mearnsii* had two peaks in litterfall, one at the end of the dry season in mid-autumn, and the other in mid-summer. *A. mearnsii* also kept a higher foliar N concentration than co-occuring native species, which gives the wattle species a competitive advantage. Native species exhibited low nitrogen concentrations which are reflected annually. In addition, the results indicated that co-occuring natives efficiently recycles nutrients before leaf abscission, for instance through high P resorption efficiencies. *Acacia mearnsii* was not as efficient in recycling nutrients, most notably N, but was more efficient in recycling P, suggesting it may require more P than can be readily supplied from the soil. The results indicate that the studied species had high resorption parameters (proficiency, *A. mearnsii* and efficiency in native species), which indicated a P limited landscape. This can be an important reason in the success of *Acacia* spp. in South African landscapes and particularly in riparian zones.

The results also indicated that *A. mearnsii* and fynbos species differed locally at all sites in instream decomposition rates, with *A. mearnsii* decaying at a much faster rate. The difference in decay rates was attributed to differences in litter quality characteristics between native and invasive species (N concentration and C:N ratio). The faster decay rates in *A. mearnsii* due to leaf litter with high N and P can have a detrimental effect on in stream functionality therefore affect the species diversity of aquatic biota. The macroinvertebrate litter affinity effects were tested and showed no preference to home turf litter or introduced littertype regardless of the local environment at each invasion status. Functional feeding groups increased at both Wit River site, as macroinvertebrates were season-dependent on

leaf litter and additionally resources *A. mearnsii* site may hold. Conversely, at the Du Toit's River low invertebrate diversity and abundances and was regulated by stream characteristics and site geomorphology at both reaches. Furthermore, seasonal hydrological regime could have accounted for macroinvertebrate species abundance and diversity at each river as there was a selective pressure on communities to utilize resources.

The research contributes to a more comprehensive understanding of nutrient cycling, acquisition and conservation strategies of native compared to invasive species in the Fynbos biome in South Africa. Additionally it also gives insight into how invading species could potentially modify aquatic ecosystems and change macroinvertebrate communities in disturbed environments. Invaders can strongly affect multiple services in an ecosystem therefore it is imperative that these mulitiple roles should be asssed and managed as environmental change (i.e, drought) could cause a long lasting effect on ecosystems holistically (riparian areas, in stream biogeochemistry and aquatic assemblages).

OPSOMMING

In hierdie studie het ek die berugte indringende wattle spesie *Acacia mearnsii* teenoor twee inheemse boom spesies in term van patrone van blare val en voedingstowwe resorpsie in rivieroewers omgewings en ontbinding proses van blare in akwatiese en makro-ongewerweldes gemeenskappe binne bergagtige strome in die Fynbos bioom van die KFO. Meer uitdruklik, die studie beoordeel: (1) blaar val patrone tussen die *A. mearnsii* en mede-voorkomende inheemse spesies op n maandelikse bases (2) die voedingstowwe (N en C) konsentrasies in blaar val maandeliks (3) die hoeveelheid voedingstowwe (N en P) wat geabosrbeer word tussen blaar spesies voor veroudering (4) en die ontbinding tariewe of proses tussen *A. mearnsii* en fynbos spesies in naby tuis omgewings en weg van n tuis omgewing om die Home Field Advantage (HFA) hipotese te toets en uiteindelik die makroongewerweldes versameling in verskillende blaar sakkies in tuis en weg van die tuis omgewings om die makro-ongewerweldes blare affiniteit binne stroom te toets.

Die resultate in die studie dui aan dat *A. mearnsii* sewe tot tien keer hoër blaar val hoeveelheid in beide die Wit en Du Toit's Rivier in vergelyking met die mede-voorkomende inheemse boom spesies binne indringende en byna ongerepte rivieroewers omgewingssone. *Acacia mearnsii* het twee pieke in blaar val, waar een voorkom aan die einde van die droe seisoen in middel herfs en die ander een middel somer in Desember. *Acacia mearnsii* het hou ook n relatiewe hoër blaar N konsentrasie as die mede-voorkomende inheemse spesies wat die wattle spesies n mededingende voordeel gee. Die inheemse spesies stal uit n laer stikstof konsentrasie wat aan gedui word maandeliks. Daarbenewens, die resultate dui aan dat die mede-voorkomende inheemse spesies doeltreffend voedingstowwe herwin voor blaar afsnyding, byvoorbeeld deur hoe P resorpsie doeltreffend te gebruik. *A. mearnsii* was nie so doeltreffend in die herwinning van voedingstowwe veral N, maar was meer doeltreffend in die herwinning van P wat aandui dat die spesie meer P vereis as wat dit beskikbaar is van die rivieroewers omgewing. Die resultate dui ook aan dat die bestudeerde spesie 'n hoër resorpsie grens het veral in vaardigheid in die *A. mearnsii* and doeltreffendheid in die inheemse boom spesies, wat aandui 'n P limitasie in die rivieroewers omgewing. Dit kan uiters die belangrikste rede wees vir die sukses van die *Acacia* spp. in Suid Afrika rivieroewers omgewings.

Die resultate dui ook aan dat *A. mearnsii* en fynbos species verskillend plaaslik by al die studie plekke in die ontbinding proses binne in die stroom gebiede met die *A. mearnsii* specie wat die vinnigste ontbind oor tyd. Die verskille in die ontbindings van die blaar spesies was aangedui deur die verskille in blaar kwaliteit tussen die inheemse en die indringende spesies (N konsentrasie en C:N verhoudings). Die vinnige ontbinding proses in

die *A. mearnsii* weens die blaar val wat hoë N en P inhoud besit kan dalk n nadelige impak het op binne stroom funksie en as gevolg van dit mag die spesie se diversiteit en akwatiese biota affekteer of beinvloed. Die makroongewerweldes blare affiniteit was ook getoets en die resultate wys geen voorkeur vir blaar tipe van sy tuis omgewing of van die blaar tiepe wat voorgestel was in die omgewing nie. Die funksionele voedings groepe het vermeerder by beide, Wit Rivier studie plekke omdat die makroongewerweldes was seisoenaal afhanklik van die blaar val asook die hulpbronne wat *A. mearnsii* indringende plekke hou. By die Du Toit's Rivier was lae nommers van ongewerweldes diversiteit en verspreidings gereguleer deur stroom eienskappe en die verskillende plekke se geomorfologie. Die seisoenale hidrologiese patron kan dalk verantwoordelik wees vir die makro-ongewerweldes spesies se verspreiding en diversiteit by beide riviere as gevolg van n selektiewe drukking deur gemeenskappe om hulpbronne te gebruik.

Die navorsing dra by tot 'n meer omvattende begrip van die voedingstowwe siklus, verkryging en bewaring strategië van die mede-voorkomende inheemse boom spesies in vergelyking teen die indringende wattle spesie *A. mearnsii* in die Fynbos bioom in Suid - Afrika. Daarbenewens gee dit ook 'n insig op hoe indringende spesies die potensiaal het om akwatiese ekosisteme dalk te verander en ook die makro-ongewerweldes gemeenskappe binne stroom. Indringende spesies kan verskeie impakte het binne 'n ekosisteem daarom is dit uiters belangrik dat die verskeie impakte moet beoordeel word en ook bestuur word. Veranderinge in omgewegings (bv., droogte) kan dalk 'n blywende negatiewe effek het op ekosisteme in 'n meer holistiese manier (rivieroewers omgewings, binne stroom biogeochemie en akwatiese versamelings).

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Introduction and literature review

1.1. General introduction

The maintenance of biodiversity is a major challenge for ecosystem management. After habitat loss, the second biggest threat to global biodiversity is invasive alien plant species (IAP's) (D'Antonio and Meyerson, 2002; Richardson and van Wilgen, 2004). The biodiversity riparian zones and rivers in South Africa are among those ecosystems most impacted by alien species across the world (Moyo and Fatunbi, 2010). In South Africa, alien invasive woody plants are particularly pernicious as they affect water resources negatively (Ghahramanzadeh, 2013). Water has been recognized as a limited natural resource which, when reaching various stages of limitation, may have the effect of crippling the South African economy (Le Maitre et al., 2002; Ashton, 2007). In the province of the Western Cape, invasive alien plants species (*Acacia mearnsii, Acacia saligna, Hakea* spp. *and Eucalyptus* spp.) in general have higher total evapotranspiration (ET) in comparison to the native vegetation, leading to declines in surface water (Meijninger and Jarmain, 2014). *Acacia mearnsii* out of all the IAP uses up to 7 mm of rainfall per day with an accumulated loss of 185 mm of rainfall used per annum (Dye and Jarmain, 2004). Therefore, the species has been earmarked as the most pervasive species of invader tree in the riparian zones of the fynbos biome in the CFR (Versfeld et al., 1998; Le Maitre et al., 2002).

1.1.1. Fynbos and invasive alien plants (IAP's) in riparian zones in the Fynbos biome of the CFR

One of the six and the smallest floral kingdoms worldwide is the Cape Floristic Region (CFR) in South Africa, which is renowned for its high botanical diversity of terrestrial vegetation (Goldblatt and Manning, 2000). The CFR is an example of Mediterranean-type ecosystems (MTE's) which is characterised by specific water availability constrains (summer drought) and nutrient availability constrains (nutrient-poor soils), which is disturbed regularly by fire events (Potgieter, 2012). The CFR vaunts a high rich diversity of plant species, which is three times greater than any other Mediterranean-type ecosystem (Cowling et al., 1992; Mucina and Rutherford, 2006). The Fynbos biome Within the CFR covers the greatest area of the three vegetation types (fynbos, renosterveld and western strandveld, Cowling et al., 1996). The fynbos vegetation within comprises approximately 9030 plant species of which 70% are common to the region (Goldbatt and Manning, 2000). Fynbos vegetation comprises

mostly of plants that are fire adapted shrub species with shallow roots (ericoid) and reed-like (restioid plants) and the soils of the biome is considered nutrient-poor (Prins et al., 2004). Fynbos plants are sclerophyllous evergreen, usually with leaves that are small, narrow and tough and generaly has very high foliar C:N ratios (Cowling et al., 1996). Plants in the fynbos region have specialized nutrient uptake and internal cycling stratagems (Powel, 2010). In the CFR nutrient cycling patterns are widespread because of the different soil types with each carrying a unique different vegetation type (Cowling et al., 1992). Availability of nitrogen and phosphorus in the soils of the ecosystem are well studied as they display different nutrient patterns and is understood to be the two elements that are likely to limit primary production of legumes, which is rare to the fynbos (Cowling et al., 1992; Potgieter, 2012). The availability of nutrients in the fynbos plays a pivotal role in the ecology of plants species in the region, their distribution and community composition (Goldbatt and Manning, 2000; Reinecke et al., 2007). Some sclerophyllous plants of the fynbos occur on stream banks and has a litterfall period that are more prolonged, which extends from summer to autumn (Maamri et al., 1994). These plants have been displaced in many riverine areas of the CFR by alien invasive plants, notably *A. mearnsii* and *Eucalyptus camaldulensis*.

The general colours of Fynbos Rivers are brown with low pH as a consequence of high polyphenolic substances seeping from dead fynbos vegetation (de Moor and Day, 2013). The dark acidic water is confined to the streams of the fynbos vegetation with pH levels as low as 3.2 recorded (Byren and Davies, 1989). Rivers in the CFR arise in the mountains and display the common profile of boulder – bed mountain streams with dense canopies. Further downstream from the dominating headwater reaches it changes to wider middle reaches with diverse cobble bed substrates and at the lower sections slow flowing reaches are found with soft bedded sand substratum characteristics (Brown and Dallas, 1995). Many rivers in the fynbos vegetation are characterised by masses of Palmiet (*Prionium serratum*) which are endemic to the region. These rivers are characterized by a seasonal variability in discharge with periods of winter flood with extreme low temperatures and summer droughts which makes the hydrological regime fairly regular (Rebelo et al., 2006). However, in recent years there are fewer wet years and more dry ones relative to 30 years ago (de Moor and Day, 2013). The stress this creates is a selective pressure for riverine freshwater species which can influence different life history traits that are synchronized with seasons that reflect summer drought and winter floods, significantly affecting invertebrate species (Bonada et al., 2007). The Fynbos Rivers in the CFR are known for the high species beta diversity of aquatic biota, particularly macroinvertebrate communities in which many remain undescribed and in most cases undetected (Wishart and Davies, 2003). As a result, the region has been earmarked as one of 200 Freshwater

Ecoregions across the world (Thieme et al., 2005). Macroinvertebrate communities are adapted to the water chemistry of the fynbos riverine systems. Waters in these mountainous regions are very pure oligotrophic, NaCl-dominated, and macroinvertebrate communities seem to disappear when the chemistry of the water is changed by the loss of organics (de Moor and Day, 2013). However, knowledge of the aquatic invertebrate communities in the river systems of the fynbos vegetation is patchy and is not as well documented as riparian vegetation studies (de Moor and Day, 2013).

The riparian vegetation is relatively unique in character from the adjacent fynbos vegetation even if it is sited under the same climatic conditions (Naude, 2012; Reinecke et al., 2007). Riparian zones are seen as the link between terrestrial and aquatic ecosystems, which encompasses of exceptional faunal, floral, soils, and extends from the edge of water bodies and ending upland on the edge of streams (Gregory et al., 1991; Naiman et al., 2005). Riparian zones can be distinguished from terrestrial ecosystems as they differ in hydrology, geomorphology and vegetation assembly (Maoela, 2015). The flora in the CFR is 66% geographically spread through the riparian areas with only 33% comprised of woody plants (Galatowitsch and Richardson, 2005; Naude, 2012). In these zone flora offers vital functions as stream bank stabilization, nutrient regulating and ecological amenities as flood mitigation (Hood and Naiman, 2000; Tererai, 2012). Fynbos vegetation comprises of tall shrubs as *Brabejum stellatifolium, Metrosideros angustifolia*, *Searsia angustifolia*, underbrush trees (herbaceous plants) and some perennial (reccurent) species below the canopy cover (Reinecke et al., 2008). Sedges and grasses are noticeable on wet bank zones and native legumes only exist in small sections under native plant cover (Power, 2010). Riparian areas support both aquatic and terrestrial communities due to the various food sources they hold which make them particularly fragile to disturbance and consequently lead to the degradation of the ecosystem (Naiman and Décamps, 1997).

The CFR has been acknowledged as a global diversity hotspot, in part due to its susceptibility to numerous processes that threatens the exceptional biodiversity of the region (Mittermeier et al., 1998). Riparian zones in the CFR are extremely vulnerable to natural disturbances such as flood rushes and fire which is known to influence riparian systems hugely (Naude, 2012; Maola, 2015). The change in ecosystem temperature, light, soil chemistry and microorganisms alters ecosystem structure and function (Reinecke et al., 2007; Richardson et al., 2007). However, the biggest threat to the biodiversity of the CFR is the persistent occurrence of invasive alien species. Invasion by introduced plants currently affect 8% of the surface area of South Africa and 29% of the Western Cape, which consist of the majority of the CFR and is the most heavily invaded of all provinces (Versveld et al., 1998). The wetter catchments in the Western Cape appear to be the area that is densest

invaded (Cowling et al., 1992). Particularly in the region of the Berg and Breede River catchments with the Breede catchment containing 84,398 hectares of invasive trees (Versfeld, 1998). However, in mountain stream sections in higher catchment areas natural riparian vegetation can still be found, but these conditions are slowly becoming non-existent (Sieben and Reinecke, 2008), with the main invaders being *A. mearnsii*, *A. saligna, A. longifolia* and *Eucalyptus* spp. in some areas (Richardson and van Wilgen, 2004). The Australian *Acacia* spp. are fast growing trees which form dense stands that dominate the canopy high line which overtops native vegetation (Witkowski, 1991a; Blanchard and Holmes, 2008). Furthermore, this enables them to out-compete shorter native species for light, which allows them to grow much taller (1 – 20 m) in a short space of time (Milton, 1981; Ehrenfeld, 2003).

Invasive *Acacia* spp. are able to persevere in invaded ecosystems through their capabilities of higher growth rates, ability to obtain nutrient and water resources (Marchante et al., 2008; Morris et al., 2011) and the capacity to accumulate larger quantities of biomass (Milton, 1981; Yelenik et al., 2007). The large quantity of biomass produces nutrient rich leaf litter and roots that penetrate deep into soil, which improve their capabilities to access a greater pool of resources (Lambers et al., 2008b; Cramer, 2010; Powel, 2010). The invasive *Acacia* spp. are adapted to sandy soils of the Western Cape as they themselves originated from the most impoverished soils in Australia (Marchante et al., 2010; Morris et al., 2011). On their root structure, like most legumes, *Acacia* spp. has N_2-fixing bacteria that allow some adaptation to the low nutrient levels in the CFR, Western Cape (Sieben, 2003, Potgieter, 2012). Therefore having the ability to outgrow and compete native species for nutrients, it is not unexpected that Australian *Acacia* spp. is renowned IAP's in South Africa (Richardson and van Wilgen, 2004; Chamier et al., 2012; Tye and Drake, 2012). This is particularly the case with *Acacia mearnsii* (DeWild) which is described by many authors as the notorious invader species along riparian zones in the Fynbos biome (e.g. Le Maitre et al., 2002; Galatowitsch and Richardson, 2005).

1.2. Nitrogen inputs from *A. mearnsii* and N-fixing IAP's into riparian zones

Acacia mearnsii, commonly known as "Black wattle", has replaced and outcompete native riparian vegetation along countless rivers (watercourses), and as consequence is ranked as the most harmful invasive species in the the Fynbos biome of the CFR (Le Maitre et al., 2002). *Acacia mearnsii* as invader specie and the impact on riparian ecosystems are well documented and researched along the rivers of the Western Cape (Crous, 2010; Le Maitre et al., 2011; Naude, 2012). Water resources are crucial to the species and as such, the *A. mearnsii* are more water dependent than other phyllodinous *Acacia* spp. in the CFR (Morris

et al., 2011). The invasion of *A. mearnsii* causes the alteration of soil chemical properties, decay rates and to an extent altering microclimates in regions they invade (Witkowski, 1991a; Yelenik et al., 2004, 2007). The species alters the nitrogen and carbon and phosphorous cycles of ecosystems (Yelenik et al.,, 2007; Naude, 2012), reduces stream flows (Le Maitre, 2002; 2011), and modifies the fire regime required for natural fynbos vegetation to reintroduce themselves (Ehrenfeld, 2003; Naude, 2012). Furthermore, the species has specialized mechanisms as extensive root systems, symbiotic N_2-fixation and nutrient conservation strategies to obtain the required resources in any environment they invade, which makes *A. mearnsii* a successful IAP's in the CFR (Yelenik et al., 2004, 2007; Morris et al., 2011).

In the Fynbos biome region, nitrogen cycling in natural environments is mainly a slow intricate process (Stock and Allsopp 1993; Yelenik et al., 2004). The slow growing sclerophyllous nature of fynbos shrubs means that nutrients recycled internally before leaf abscission (Norbly et al., 2000). Studies have found that natural fynbos vegetation have lower rates of leaf litterfall, with low levels of N concentrations, high C:N ratio's, slower rates of decomposition than the N_2-fixing plants (Witkowski, 1991a; Allsopp and Stock, 1993). Ultimately, the smaller quantities of N in fynbos plant species do not deposit a rich amount of nitrogen to contribute to an overall impact on soil status of a region, thus making them a lesser roleplayer in ecosystem processing (Yelenik et al., 2004). Invasive alien N_2-fixing *Acacia* spp., on the other hand, can form dense evergreen monocultures that regenerate after every fire regime (Milton, 1981; Yelenik et al., 2004). The IAP's trees are much taller than the native counterparts and produce considerably more biomass than native species but specifically the *Acacia* spp. (Milton, 1981; Witkowski, 1991a; Yelenik et al., 2007). In a study done by Milton (1981) in the Southern Western Cape it was found that the biomass of *Acacia* spp. (*A. saligna, A. cyclops, A. longifolia* and *A. melanoxylon*) stands are about ten times greater than those of fynbos vegetation. Hence, nitrogen concentrations of the leaves in these Australian *Acacia* spp. found in the Milton (1981) study were 2 - 4 times greater than the fynbos plants. In a later study in terrestrial regions (Melkbosstrand and Malmesbury) in the Western Cape, Witkowski (1991a) reported that *A. saligna* and *A. cyclops* had higher litterfall production than the two comparable fynbos plants (*Leucospermum parile* and *Pterocelastrus tricuspidatu*s). In the same study, higher N concentrations were found in the leaf litter of the invader species, which resulted in higher levels of total N (Witkowski, 1991a). In both the Milton, (1981) and Witkowski, (1991a) studies the highest N concentrations were found in the leaf litterfall of the invasive species and not in other plant components. In a similar area in the Riverlands Nature Reserve, Western Cape, Yelenik et al., (2004) found that the litterfall of *A. saligna* was three times higher than fynbos vegetation and similarly had

a nitrogen concentration that was almost 3 times higher than the fynbos species. Likewise, in a recent study by Naude, (2012) in different river systems within the south-western Cape region invaded sites (of *A. mearnsii* and *A. longifolia)* showed a litterfall rate that was twice as much as the fynbos vegetation in the study. Nevertheless these estimates of litterfall were only calculated annually (Milton, 1981; Yelenik et al., 2004; Naude, 2012) or bimonthly (Witkowski, 1991a) and remains relatively unknown in riparian areas in the Fynbos biome.

High foliar N concentrations have been reported in other studies on *Acacia* spp., particularly *A. mearnsii*. In a study within the south-western Cape Province in various riparian systems by Maoela (2015) and Tye and Drake (2012) in the Komati River, Mpumalanga found elevated N concentrations in *A. mearnsii* compared to native species. Juba (2012) in an unpublished research study reported high N concentrations in *A. mearnsii* and low N concentrations of the native species in the riparian zones in the Western Cape. The study compared leaf nutrient stocks between *A. mearnsii* and two co-occurring native species (*B. stellatifolium* and *M. angustifolia*). Most recently Van der Colff et al., (2017), in a study in the Western and Eastern Cape province (Garden Route National Park) found that *A. mearnsii* had high levels of N concentrations in its leaves which can increase the amount of N entering the environment (Yelenik et al., 2004, 2007; Morris et al., 2011). These key traits of communities of *Acacia* spp., of larger size and much higher inputs of N-enriched litter with rapid turnover rates may play an important role in enhancing N cycling and concentrations in terrestrial areas and riparian corridors. Consequently this can lead to more N in leaf litter returned to the soil fo the riparian zones and eventually transferred to aquatic environments. The ^{15}N natural abundance technique can shed some light on cycling of N, especially regarding the openness of the N cycle (Robinson, 2001; Fry, 2006). As an integrator of the N cycle, the ^{15}N natural abundance can also be an indicator, and viewed in conjunction with other indicators such as N stocks and decomposition, used to infer the magnitude of fluxes of N.

1.2.2. Resorption efficiencies by N_2-fixing and non-fixing IAP's

A central component in plant communities is the uptake, processing and conservation of nutrient resources (Craine et al., 2009). Nutrients in plants accumulate as part of the cycling of resources between plants, soil and the atmosphere and are the most essential process in nutrient dynamics (He et al., 2011). In ecosystem nutrient cycling, nutrient allocation and conservation strategies play a major role in native plant communities. Habitats that are nutrient poor typically have species with nutrient conserving strategies, which is a crucial dynamic in nutrient deficient environments (Aerts, 1995; He et al., 2011). There are several plant mechanisms that entail strategies to conserve nutrients such as plant material with

long lifespans and low tissue nutrient concentrations (Wright and Westoby, 2003; Zhang et al., 2014). Plants can also conserve nutrients in an active physiological strategy called nutrient resorption whereby they remobilize limiting nutrients prior to leaf abscission (Wright et al., 2004). The resorbed nutrients during senescence are immediately available for the plant for growth tenacities and reduce the plants reliance on instant nutrient uptake (He et al., 2011). The degree of nutrient resorption can play a significant role on soil nutrient availability, as the nutrients that are resorbed generally end up in leaf litter fall, which decomposes and becomes available for the plant to take up again (Aerts, 1995; Aerts and Chapin, 2000). Perennial plants are partially dependant on internal nutrient cycling and the capacity to absorb nutrients are important features in the fitness of the plant species especially in nutrient poor ecosystems (Aerts et al., 2007; He et al., 2011; Tye, 2013).

Two important nutrients for plant growth are Nitrogen (N) and phosphorus (P) which is generally restrictive for plant growth in natural surroundings (Jacobs et al., 2006; He et al., 2011). For places like southern Australia and South Africa this is particularly true, which is either N or P, limited as these regions have highly weathered soils (Lambers et al., 2008b). To overcome N and P limitations plants in these regions overtime developed mechanisms to overcome these limitations and one of these mechanisms is nutrient retranslocation (resorption of N and P) by which scarce nutrients may be recouped (Lambers et al., 2008b; Potgieter, 2012). The general hypothesis is that N is mainly supplied by the ecosystem through N_2-fixing symbioses (Chapin et al., 2002) and P is nutrients derived from rocks, due to mineral weathering as soils in these landscapes such as the Fynbos biome are generally characterized as acidic (Rebelo et al., 2006; Powel, 2010; Potgieter, 2012). Nutrient stocks especially P, contribute little to ecoystems in the Fynbos biome as P content in soils range between 0.0003 and 0.2 mg P g^{-1} (Potgieter, 2010). Plants are seen as generally being more efficient at P resorportion than N resorption with the global averages according to Aerts (1996) being 52% for P and 50% for N, and this likely to be the case in the Fynbos biome.

Little literature can be found about Acacia spp. in nutrient impoverished Mediterranean environments as the CFR. Nutrient allocation patterns in *Acacia* spp. have been found to differ between seasons in a broad spectrum (Tolsma et al., 1987). Yet, information on nutrient allocation of the species is scarce in literature. Leaves receive the most attention as it is easy to sample and known for its importance in plant productivity and high turnover rates (Tolsma et al., 1987). In the aboveground component of *Acacia* spp., leaves tend to have elevated concentrations of N and P (Witkowski, 1991a; Caldiera et al., 2002). Chlorophyll, ATP and other metabolic compounds are essential for plant productivity and enriched with N and P, which leads to high leaf N and P concentrations (Sterner and Elser, 2002). Many Australian *Acacia* spp. have high N concentrations (Witkowski, 1991a;

Yelenik et al., 2004, 2007; Tye and Drake, 2012) in comparison to non-N fixing species (or even African *Acacia* species), due to the N_2-fixation strategies of the invasive *Acacia* species. In many African acacias, the commonly perceived trend in dry months is the translocation N and P out of senesced leaves (Tolsma et al., 1987). In some studies on *Acacia* spp. (Witkowski, 1991a; He et al., 2011; Van der Colff et al., 2017) higher P vs N resorption efficiency are found, which has been put forward to be a consequence of P limitations in native environments. Plants in the native families *Proteaceae* and others are well represented on P-impoverished soils and are often seen as keystone species (Crous, 2010), which contains cluster roots. These plant species do exist in the Fynbos biome and are better adjusted to access soluble P in these ancient highly weathered soils (e.g. through proteoid roots, cluster roots) than plants such as the *Acacia* spp., which form mycorrhizal symbioses (Lambers et al., 2008b; Lambers et al., 2010). Therefore, native species effectively mine P that is unavailable for plant through their cluster roots, which makes them good conservation strategist for the oligotrophic soils of fynbos environments.

However, the most puzzling question in the Fynbos biome is how does the *Acacia* spp. satisfy their demands for P particularly in a region such as the Fynbos biome?

Australian *Acacia* spp., possess extensive root systems and mycorrhizal symbiosis, which allows them to enlarge the soil volume and increase the number of places for mycorrhizal establishment to enhance acquisition of P and other nutrients (Hoffman and Mitchell, 1986; Power, 2010). P-acquisition through synergetic nitrogen fixation has a high demand for P as up to 20% of plant P is distributed to nodules (Stock and Allsopp, 1992; Schulze et al., 1999, Potgieter, 2012). In the south-western Cape Witkowski (1994) found that *A. saligna* root penetration was faster than *A. cyclops* over a month period, which potentially could have assisted them in tapping into the water table, and also might have assisted with nutrient aquisition. Cramer et al., (2009) stated that greater water availability could contribute to P–acquisition via mass flow. Additionally, these adaptations to satisfy their water and P demands, such as the use of deep, extensive root system and symbiotic association with mycorrhizal fungi most likely contribute to their success in the Fynbos biome. However there is a considerable gap in knowledge surrounding the resorption efficiency N and P in IAP's in nutrient impoverished ecosystems such as the Fynbos biome of the CFR (Diaz, et al., 2012; Potgieter, 2012) as the majority research are done on temperate forests and wetland ecosystems (Morris et al., 2011).

1.3. Decomposition, home field advantage effects and the role of freshwater invertebrates on decomposition in natural and invaded streams

Leaves decompose at different rates in aquatic environments (Petersen and Cummins, 1974) which is dependant on both internal and external factors (Webster and Benfield, 1986). The internal factors are mainly the difference in leaf litter inputs (C, N and P) and structural properties as leaf shape as well as the composition and abundance of the macroinvertebrate communities (Webster and Benfield, 1986; Reinhart and VandeVoort, 2006). The external factors are stream characteristics (temperature, flow regime, physical abrasion, and substrate) which is different in upstream and downstream reaches and different microhabitats (pools, runs and riffles) within a stream ecosystem and therefore different decay rates are found (Sponseller and Benfield, 2001; LeRoy et al., 2006). There are a few studies (King et al., 1986; 1987) in the Fynbos biome assessing the effect of environmental conditions on litter breakdown; however, to unravel the effects are not easy as there are variation in site characteristics, litter quality differences between species and macroinvertebrate communities in stream (Bengtsson et al., 2011). Therefore, the processes and factors influencing decomposition in freshwater environments in the Fynbos biome mountain streams remain sparse.

Inputs from leaf litter from different plant species are different in structure and chemical properties such as leaf shape, N concentrations, carbon: nitrogen ratio (C:N) and lignin concentrations (Aerts, 1997; Gholz et al., 2000; Ayres et al., 2009). These inputs are seen as a major vector moving energy and nutrients for freshwater biota within aquatic ecosystems (fungi, bacteria, and macroinvertebrates) (Negrete-Yankelevich et al., 2008; Ayres et al., 2009; Kuglerova et al., 2017). The physiological factors mentioned explain up to 70 % of the disparity in leaf litter decomposition and the additional 30 % by in stream characteristics and HFA effects (Gholz et al., 2000; Parton et al., 2007). In riparian zones the invasion of alien invasive plant species (IAP's) is normally connected with modification of aquatic environments (Braatne et al., 2007) due to the quality and the quantity of leaf litter inputs (Boyero et al., 2012). These modifications often convey substantial changes in ecosystem function and macroinvertebrate communities (Levine et al., 2003; Boyero et al., 2012). In the last ten years studies (Ehrenfeld, 2003, Allison and Vitousek, 2004) found that invasive alien plant species which has high leaf litter composition (especially N and P) tend to decompose much faster than native species.

Faster leaf litter decomposition from IAP's incomparison to native species have been reported when the invasive plants were N_2-fixing and the native species not (Witkowski, 1991a). The physiological characteristics of IAP's of high nutrient concentrations, species

specific leaf area (SLA) and N_2-fixing capabilities are key functions in faster decomposition rates when compared to native species (Allison and Vitousek, 2004; Morris et al., 2011). In contrast, slower decomposition rates of IAP's were also found in some studies (Witkowski, 1991a; Drenovsky and Batten, 2007).

There is a growing amount of evidence that plant species have species specific or affinity effect to certain macroinvertebrate communities (Veen et al., 2015). The decomposer communities as a result may become adapted to and form a specialized affinity to the litter they encounter over an extended period. As a result they become more efficient at breaking down their own litter matrix, e.g. from the riparian plant community above them (Ayres et al., 2009). Consequently this "at home" benefit has been referred to as the 'home-field advantage' (HFA) hypothesis where leaf litter in its home environment decomposes faster in its native or home site than away from it (Gholz et al., 2000; Ayerez et al., 2009). The specialized affinity effect macroinvertebrate communities has on certain litter types driven by interacting drivers such as the different leaf litter quality received as input from the riparian zone and the incubation conditions which can be measured over weeks or months (Jewel et al., 2015; Veen et al., 2015).

In literature there is glut of evidence (Freschet et al., 2012; Veen et al., 2015) which tested for, but did not show the occurrence of HFA, hence the conditions under which it exist is uncertain. Litter diversity in a home environment is not always associated with faster decomposition rates and macroinvertebrate litter affinity effects (Austin et al., 2014; Veen et al., 2015). Evidence of HFA effects where invertebrate decomposer communities become adjusted to feeding on their home turf litter but are less efficient at breaking down the foreign litter regardless of plant diversity or C:N ratios (Veen et al., 2015). The difference in effects is variable, depending on both biotic and abiotic factors of the ecosystem, which influences litter decomposition rates (Veen et al., 2015). It remains relatively unknown if disturbance events such as the introduction of alien invasive plants species (IAP's) can affect macroinvertebrate litter affinity effects. For example, the invasion of N_2-fixing plants could change aquatic environments due to chemical and physical traits differences to leaves from native vegetation (Morris et al., 2011). To have a better understanding how and when decomposition rates and macroinvertebrate communities interrelate with litter to influence HFA effects, it is crucial to investigate the significant drivers of the interaction between the plant community, litter type and environmental conditions at present (Freschet et al., 2012). Up till now, it remains uncertain if litter of a different type would affect macroinvertebrate communities in a HFA microsite as vegetation peaks (litterfall period) generally follows a decline in invertebrate species richness (Buddle et al., 2006) therefore these mechanistic links needs added investigation (Van der Wal et al., 2013).

The replacement of native riparian tree species with IAP's in the Fynbos biome is likely to affect and modify aquatic habitats of macroinvertebrate communities in adjacent streams (Richardson and van Wilgen, 2004; Reinhart and Vande Voort, 2006; Samways et al., 2011). Despite the well-documented information of the invasion of *A. mearnsii* on terrestrial communities, there is little or no information of the effects the species invasion leaf processing and macroinvertebrate communities in stream. Only previous work of Lowe (2008) and Samways et al., (2011) looked at the highly endemic aquatic macroinvertebrate communities of the CFR and the effect invasion of particularly *A. mearnsii* has on them and by King et al., (1986;1987) on native fynbos species.

1.4. Control and management of IAP's in riparian zones

Many river, streams and adjacent ecosystems in South Africa are impacted by *A. mearnsii* invasion. Large-scale control efforts in areas affected by *A. mearnsii* and other IAP's are instigated by Working for Water (WfW) programme of South Africa. The Department of Water and Sanitation, formerly known as the Department of Water Affairs and Forestry established the Working for Water (WfW) programme in 1996. The programme in South Africa is the leading government funded stream restoration programme has spent close to an R100 million on controls and eradication of alien invasive plants nationwide (van Wilgen, et al., 2001). The restoration programme declared that, ± 2 million hectares containing alien invasive plants by 2015 would be cleared (van Wilgen et al., 1998, 2011). The program also promised job delivery for local people. The techniques used to remove IAP's like as *A. mearnsii* IAP from fynbos vegetation are mainly done by felling, removal of biomass, with slash and burn as another option, which is a controlled process (Stock and Lewis, 1986). Alien invasive plant species clearing plays an important role in the recovery or delay of native riparian area plant communities (Holmes et al., 2008). Nevertheless, removing IAP's can create further disruption on riparian areas and adjacent stream aquatic environments and surface water bodies (Samways et al., 2011) while long-lasting effects on ecosystems process may also still influence restoration. Many researchers have the view that changes in nutrient status could have a negative affect on the re–establishment of native plant species because of clearing, due to nutrients that consequently remain in soil of riparian systems for an extended duration period, the so-called legacy effect (Brown et al., 2004; Yelenik et al., 2007; Naude, 2012).

1.5. Research aim, objectives, hypotheses and key questions

1.5.1. Overall aim

The purpose of the present study is to quantify and compare the N inputs and decomposition rates in near pristine and invaded sites between co-occurring native species (*Brabejum stellatifolium* and *Metrosideros angustifolia*) and the invasive alien species *Acacia mearnsii* in catchments areas of the Breede Water Management Area (WMA). Importance is placed on the riparian zones of these sites by looking at leaf litter inputs from both *A. mearnsii* and co–occurring native species and the different rates of decomposition.

1.5.2. The objectives of this study were to:

A. To quantify the amount of leaf litter inputs on the riparian zones by *A. mearnsii* and co-occurring native species in near pristine and invaded riparian zones;
B. To determine the N and C concentrations in aboveground components in different seasons (summer, autumn, winter and spring) between *A. mearnsii* and co-occurring and to determine the N return to soil in leaf litter inputs by *A. mearnsii* and co–occurring native species in near pristine and invaded riparian zones;
C. To determine the resorption efficiencies (N and P) between *A. mearnsii* and co-occurring native species on near pristine and invaded riparian riparian zones;
D. To determine the instream decomposition rates between *A. mearnsii* and co-occurring native species and to determine if home field advantage (HFA) plays a role in decomposition rates when litter is placed far from origin;
E. Identify and determine the diversity (species richness, abundance) of macroinvertebrates of *A. mearnsii* and co–occurring native species in their native reaches and to determine if HFA plays a role in macroinvertebrate communities affinity affects when litter is placed far from origin;

1.5.3. The hypotheses of the study are:

A. *A. mearnsii* shed more leaf litter annually than co-occurring native species in riparian zones;
B. *A. mearnsii* has higher annual N concentrations but lower C:N concentrations in aboveground components in different seasons compared to the co–occurring native species;
C. The co–occurring native species has higher nitrogen resorption efficiencies (NRE) and phosphorous resorption efficiencies (PRE) than *A. mearnsii* in the riparian zones;

D. *A. mearnsii* decomposes at a faster rate than co-occurring native species in both near pristine and invaded reaches;
E. Macroinvertebrate communities are abundant in invaded compared to near pristine reaches and macroinvertebrates communities and has no preference for either littertype

1.6. Organisation of thesis

This thesis presents findings on nitrogen inputs from invasive *Acacia mearnsii* in comparison to two native species into riparian zones and aquatic environments in the Cape Floristic Region, South Africa, specifically at the Wit River (Bainskloof Pass) and the Du Toit's River (Franschhoek Pass, Franschhoek). The thesis contains five chapters. **Chapter 2** is a detailed overview of the site study areas and the methodology used in responding to the objectives. **Chapter 3** investigates the N inputs of *Acacia mearnsii* and co–occurring native species. The chapter is based on the monitoring and measurement of leaf litterfall inputs and seasonal N and C concentrations, and N resorption in pristine and invaded sites in the Western Cape. The first 3 of the 5 objectives are addressed in this chapter. **Chapter 4** investigates the decomposition rates of *Acacia mearnsii* and co–occurring native species and macroinvertebrate communities in leaf litter bags in near pristine and invaded reaches. The chapter focuses on decomposition rates of *A. mearnsii* and the two co–occurring native species, which is mixed in one leaf litter bag ('fynbos species'). The last 2 of the 5 objectives (listed d and e) are addressed in this chapter. **Chapter 5** summarizes and integrates the major findings from the studies presented in Chapters 3 and 4. The research and results in the study would further contribute to the restoration of fynbos riparian zones as well as to close the gap in the limited knowledge of nutrient dynamics of *A. mearnsii* and its invasiveness on riparian zones and the impact on aquatic environments in the Fynbos biome of the CFR. It should be noted that the the two data chapters (3 and 4) are written in the form of manuscripts for submission to journals, thus some duplication, especially with regards to materials and methods are unavoidable, especially in relation to Chapter 2.

Chapter 2

Detailed description of study species and sites

2.1. Studied species

2.1.1. *Acacia mearnsii*

Acacia mearnsii, also commonly referred to as Black wattle is a native plant species originally from Australia where it is prevalent on the south eastern side and in Tasmania. In South Africa the species was introduced in 1864 as a windbreak as well as for shade, fuel sustenance and the tannin content of its bark (Le Maitre et al., 2011). *Acacia mearnsii* is a member of the family Fabaceae and subfamily Mimosoideae (Moyo and Fatunbi, 2010). The species is an evergreen species which occurs in majority altered river systems in South Africa (Versfeld et al., 1998; Moyo and Fatunbi, 2010). *Acacia mearnsii* is often prevalent at river systems in the Fynbos biome along riparian zones (Moyo and Fatunbi, 2010; Maoela, 2015). Particularly in the Western Cape where the species has replaced countless native riparian species by outcompeting them due to these trees developing dense, evergreen monoculture thickets (Richardson et al., 2000; Le Maitre et al., 2002).

The species outcompetes native plants for water, nutrients through physical mechanisms such as a sophisticated root systems (extensive and deep), symbiotic N_2-fixation and is known to be drought tolerant (Crous, 2010). Areas invaded by *A. mearnsii* are reported to have a greater leaf litter mass than uninvaded areas which inhibits the formation of native seedlings (Witkowski, 1991a; Moyo and Fatunbi, 2010). The infestation of the species alters microclimates, litter decomposition rates, and soil physical properties. These potential impacts of the invaded species often occur due to difference in plant structure and chemical composition of leaf litter when compared to native vegetation leading to an altered biogeochemical and physical environment (Morris et al., 2011). When comparing *A. mearnsii* to other *Acacia* spp. (*A. cyclops, A. saligna* and *A. longifolia*) in the Fynbos biome, *A. mearnsii* is the tallest growing acacia species growing to heights of up to 20 m and forming thick stands and maintaining a high green leaf capacity yearly.

Acacia mearnsii tree has leaves that are bipinnate, which turns a brown colour during dry conditions (Crous, 2010; Naude, 2012). The species is predominately found in cooler mountain reaches and grows in disturbed mesic habitats and flourishes on poor dry soils but favours moist, fertile deep soils (Witkowski, 1991a). The ability to produce enormous amount of seeds is partly implicated in the invasiveness of the species in a habitat. The seeds lie

dormant and are triggered through bush fires which allow them to germinate and ultimately develop a large crown that shade out other native species (Moyo and Fatunbi, 2010). Based on estimates pods normally contain on average seven seeds with an maximum seed production of between 48,600 and 70,200 seeds per kilogram from the fifth year onwards (Moyo and Fatunbi, 2010). The black wattle species is hermaphroditic; flowers with bees being the main pollinators and is said to flower from September to November (Campbell, 2000; Nyoka, 2003; Dell'Porto, 2006), however, some communities have shown irregular periods of flowering patterns in Australia (Moncur et al., 1988; Bonney, 2003). *Acacia mearnsii* has all the attributes to becoming a successful invader; in addition to the characteristics mentioned above, it also has a short juvenile phase, decent yearly seed crop and small seeds dimension which can persist in the soil for more than 60 years (Pretorius et al., 2008) (Figure 2.1a).

2.1.2. *Brabejum stellatifolium*

*Brabejum stellatifolium (*wild almond) is a member of the Proteaceae family and is the only member of the genus Brabejum (Smith, 1966; Rourke, 1971, Figure 2.1b). *Brabejum stellatifolium* trees are limited to the Fynbos biome in the Western Cape and are found growing alongside river corridors on the lower slopes of valleys from Clanwilliam to the Hottentots Holland Mountains (Crous, 2010). They are also abundant on the Cape Peninsula and the eastern side of Table Mountain. The species is an evergreen tree, which is often large in size (5 m tall) with a wide spreading multi-stemmed shrub (Smith, 1966). The leaves of the plant are dark green in colour, rubbery to touch and is shaped in a sharply lance shape toothed leaf with a prominent vein along the midline of the leaf (Rourke, 1971; Jackson, 1990). The leaves are arranged in whorls of six at intervals along the stems, radiating out like a star around the branch. The flowering time for *B. stellatifolium* is around mid-summer (December – January). The fruits of the plant are carried in a cluster at the tip of branch and look similar to almonds and are densely covered with hairs that are a velvet chocolate-brown colour. The wild almond species are pollinated by insects when they flower and fruits float and are dispersed by water. They are also short-lived and die in storage on the riparian area (Coates-Palgrave, 2002). The fynbos biome is fire susceptible, and the wild almond survives the normally quick fynbos fires by resprouting from the stem (Rourke, 1971).

2.1.3. *Metrosideros angustifolia*

Metrosideros angustifolia (Cape gum) is the only indigenous member of the Myrtaceae family in the Fynbos biome (Goldblatt and Manning, 2000). *Metrosideros angustifolia* is an evergreen plant which can occur as a multi-stemmed, spreading, perennial shrub or small tree. The species can reach heights of 2 – 7 mm with a V-shaped canopy that is dense (Palmer and Pitman 1972; Goldblatt and Manning, 2000). The twigs and petioles are often shaded pink with the bark of the tree being grey-reddish brown and flaky at times (Goldblatt and Manning, 2000). The leaves are thin and leathery with narrow smooth margins. When held up in bright light tiny glands are visible as dots on the surface (Thomas and Grant, 2008). The flowers are small and fluffy and a cream to pale yellow colour form late in spring (November) until late summer (February). The cup–shaped capsules are broad–ranging, containing up to three seeds. Once ripened these capsules split open and release their seeds (Coates-Palgrave, 2002). The fruit and seed of this tree attract birds and animals. The flowers are pollinated by various insects and are visited by bees (Milewski and FitzPatrick, 2006). The gum species grows in mountainous areas of the Cape and is indigenous to the Fynbos biome (Milewski and FitzPatrick, 2006). The plant species can be found along areas in the western part of the south coast where there is increased moisture and it also grows where it is naturally wet, in the acidic, sandstone and stream gravel regions of the Western Cape (Coates-Palgrave, 2002; Milewski and FitzPatrick, 2006) (Figure 2.1c).

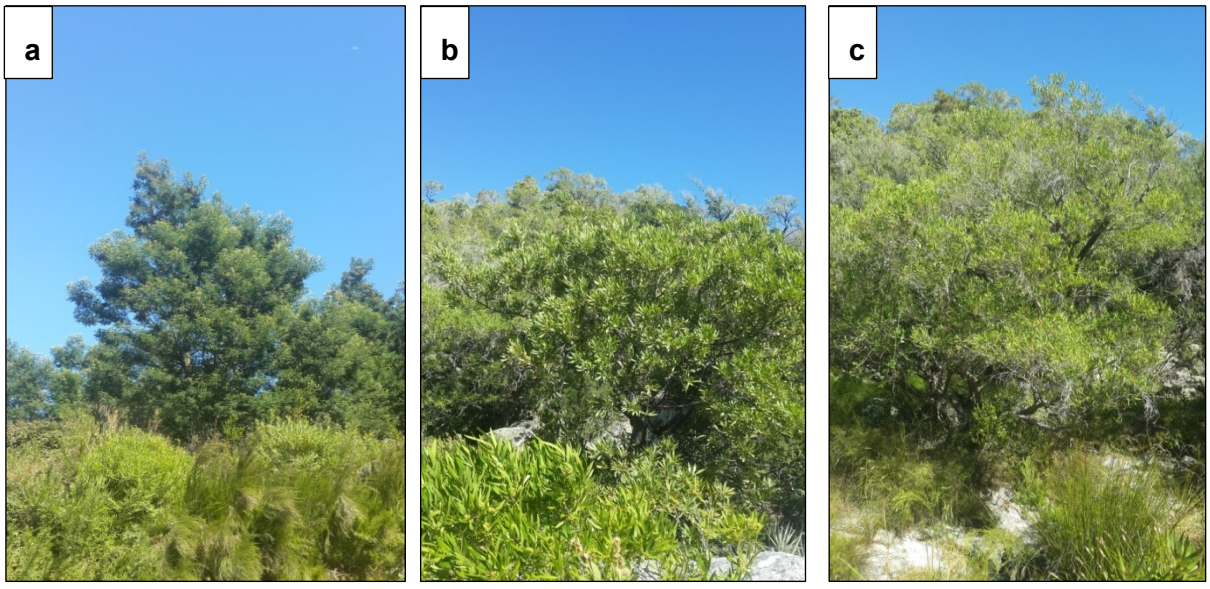

Figure 2.1: Tree species of *Acacia mearnsii* (**a**), *Brabejum stellatifolium* (**b**) and *Metrosideros angustifolia* (**c**).

2.2. Description of study sites in the Fynbos biome of the Cape Floristic Region

The study was carried out in the Western Cape from January 2016 until February 2017 in the mountain streams and mountain transitional zones of the Mediterranean-type Fynbos biome (Reinecke et al., 2013) in the Breede Water Management Area (WMA) (Figure 2.2). The sample sites/reaches were chosen based on four criteria: Firstly, sites were based and chosen on the presence of the two native plant species that are naturally confined to the fynbos biome (Richardson et al., 1992; Thuiller et al., 2006b) and the invasive plant species. Secondly, study sites should have no commercially afforested or agricultural areas. Thirdly, sample sites were arranged so that fynbos sites were upstream followed by alien sites downstream of fynbos sites. Fourthly, sites were chosen based on their history of *Acacia*-invasion and the condition of the fynbos vegetation (Figure 2.3 a, b). A sample reach had to consist of fynbos vegetation, which was the reference sites (here after, near pristine site), and a heavily invaded area (here after, invaded site) with an area cover of at least 50% (*A. mearnsii*, dominated site). The study area is characterized by dry summers and wet winters. After the first heavy rains, mountain streams and rivers in the CFR have ample surface flow in winter and in summer many streams are reduced to perennial pools and section of riffles resulting in periodic water shortages (Goldblatt and Manning, 2000; Rebelo et al., 2006). In these catchments the prevailing lithology is the quarzitic Table Mountain Sandstone of the Peninsula formation (Rebelo et al., 2006). Soils in the catchment produces shallow coarse surfaced soils and is mainly characterised by soils which are nutrient poor, leached and acidic (Lambers et al., 2010).

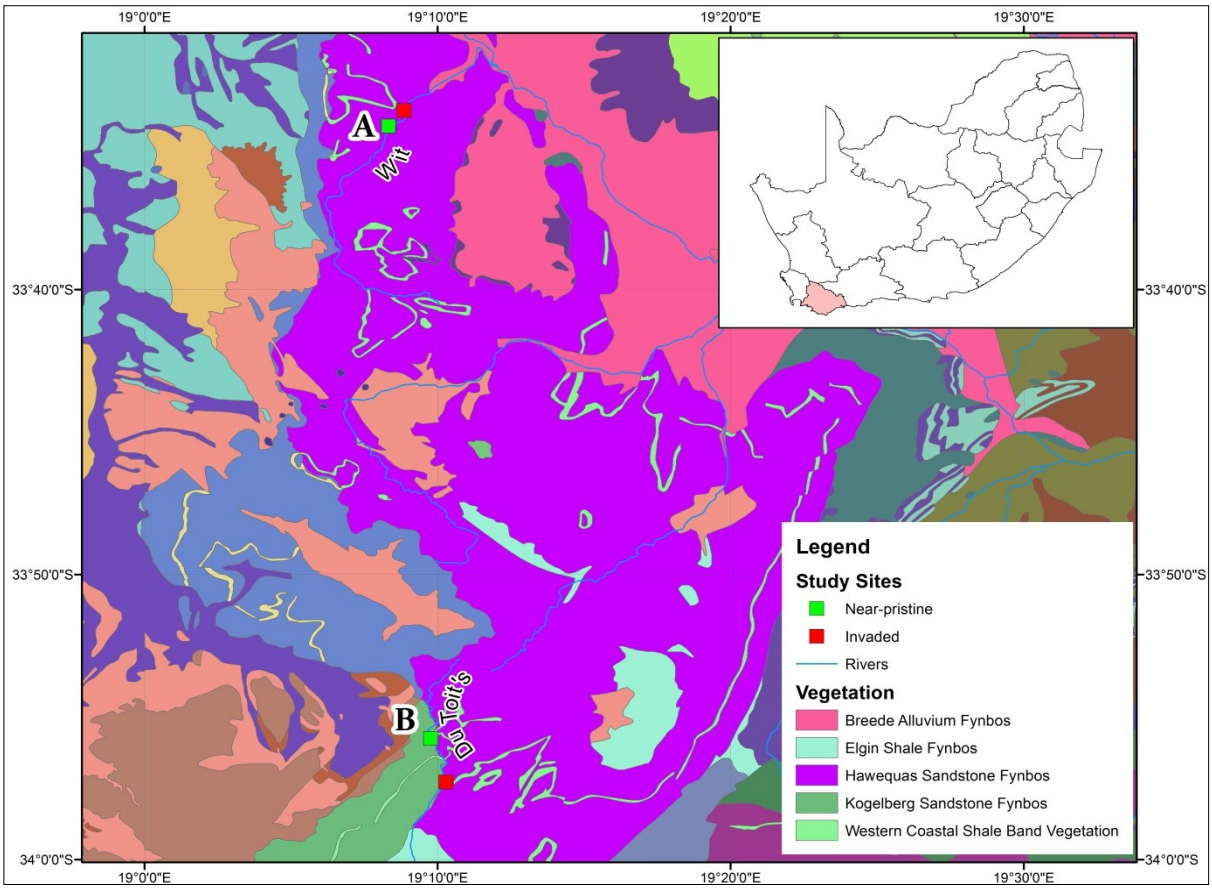

Figure 2.2: Location of the two perennial rivers in the Western Cape, Breede Water Management Area (WMA): Wit and Du Toit's River, and the two invasion status (green: near pristine; red: invaded site) at each river.

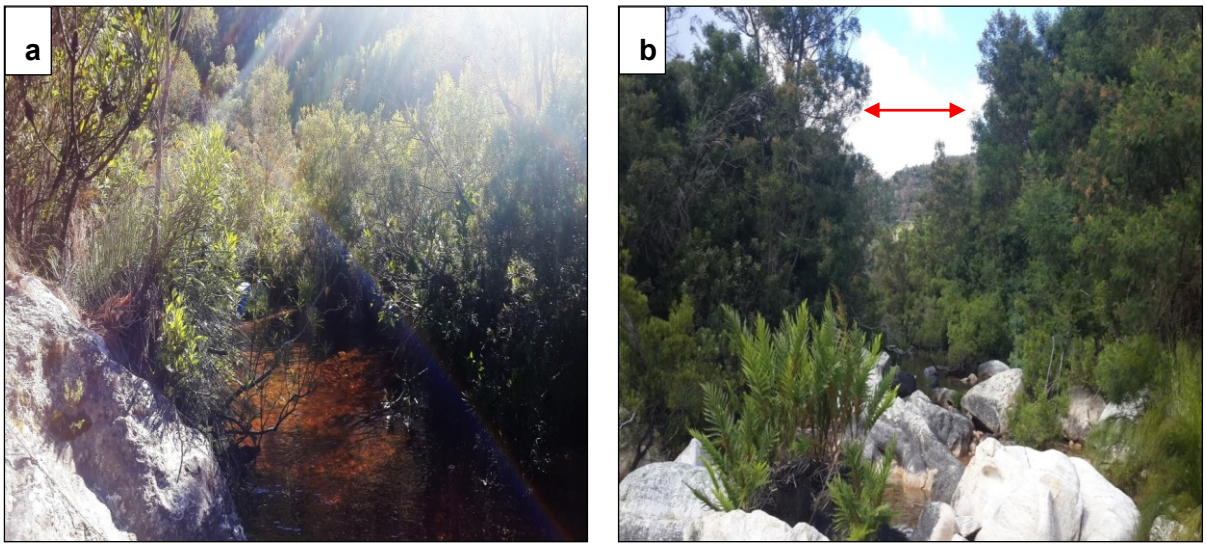

Figure 2.3: Photographs of the different invasion treatments: (**a**) near pristine (NP), and (**b**) invaded site (IV). The red arrow indicates invasion by *A. mearnsii*.

2.2.1. Wit River (Bainskloof Pass)

The Wit River is a small perennial tributary of the Breede River which is approximately 11 km long and arise in the Bainskloof Pass, Hawequas mountain range (Rebelo et al., 2006; Maubane, 2016). The Wit River is drained by Slanghoek Mountains on the western slopes and the Obiekwa Mountains in the south (Reinecke et al., 2007; Naude, 2012). The catchment geology of the Wit River comprises of the Peninsula formation which supports the Hawequas Sandstone Fynbos vegetation type (Rebelo et al., 2006). The area receives an annual average rainfall of 1200 mm, which occurs mainly between May – August (Rebelo et al., 2006).

The near pristine site at the Wit River was located above the Tweede Tol camp site. The sample reach was approximately 140 m in length and 10-16 m wide through the channel. On the dry leftside bank looking downstream, the dominant vegetation was characterized by *B. stellatifolium*, *M. angustifolia, Morella serrata* and some scattered individuals of *Erica caffra* and *Elegia capensis*. On the right bank close to the water's edge *Prionium serratum* (Palmiet) were found throughout the reach and many *B. stellatifolium*, *M. angustifolia* and *Brachyleana neriifolia* individuals scattered along the channel and up the mountain foothill. The plants species mentioned were all described by Campbell (1985) as the Wit River riparian community (Figure 2.4 a, b). The invaded site was 1.6 km away from the reference site downstream. The reach in the invaded area is approximately 210 m in length and 8-14 m wide throughout the channel. Upstream, the lateral zones of both the wet (left) and dry (right) banks were heavily invaded by stands of *A. mearnsii* trees. *Acacia mearnsii* stands were dominant throughout the riparian zone on the right side with the canopy distinctively noticeable from the side of the road. Only a few scattered individuals of *B. stellatifolium* and *M. angustifolia* were evident between *A. mearnsii* trees. On the left bank *B. stellatifolium* and *M. angustifolia* were more scattered than on the right bank, growing close to the water's edge and under closed stands of *A. mearnsii* trees which shaded the stream in certain sections.

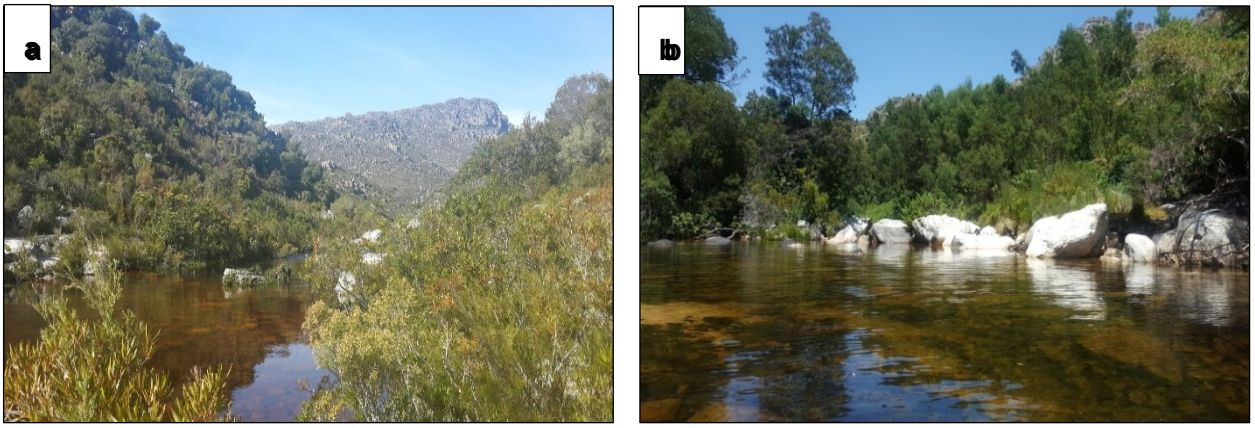

Figure 2.4: Near pristine (**a**) and invaded site **(b**) at, the Wit River. Photographs were taken in summer (December 2016).

2.2.2. Du Toit's River (Franschhoek Pass)

The perennial Du Toit's river forms part of the Breede system, but is in essence a tributary of the Riviersonderend River, which is shortened by the Theewaterskloof Reservoir (Tharme and Brown, 2004). The Middagkransberg and Franschhoek Mountain is the source of the river, at an altitude of about 1500 m (Wu, 2005). The surrounding mountains of the Du Toit's river are made up of quartzite sandstone and thin bands of shale and conglomerate (Wu, 2005). The Du Toit's River is approximately 13.4 km in length and flows throughout the steep–sided and narrow Franschhoek Pass under a small bridge before flowing through a large wetland (Palmiet dominated) and into the Theewaterskloof Reservoir. Mountain fynbos covers the best part of the area, with no agricultural or commercial areas. The Du Toit's River receives annual average rainfall of 1200 mm, which occurs mainly between May – August (Rebelo et al., 2006).

The near pristine site is located in the mountain stream section of the Du Toit's River 2 km downstream from the Mont Rochelle Nature Reserve. The sample reach was approximately 110 m in length and 4–7 m in width consisting of a single-narrow channel with short cascade sections with shallow riffles. Looking downstream, the sample site had generally well-developed native riparian fynbos. On the left dry bank, looking downstream, the bank was dominated by *B. stellatifolium* and tall *M. angustifolia* which in some parts of the channel were hanging over into the stream (Figure 2.5a). Up on the mountain foothill section, *B. stellatifolium* species were scattered with small grasses in between. The aquatic zone in stream and along the channel margins was dominated by palmiet (*P. serratum*) and sedges (*Isolepis digitate*) on the top of immovable small boulders and bedrock.

The invaded site was 3.15 km downstream from the near pristine site close to the palmiet-dominated wetland and far away from the Theewaterskloof Reservoir (Tharme and Brown, 2004). The reach was approximately 130 m in length and 10–16 m wide throughout the channel. The riparian area on the right bank, when looking down stream, was dominated with *A. mearnsii* stands. Stands of young *A. mearnsii* trees were growing on the side of the riparian area and older dense thickets were found further downstream on the riparian area (Figure 2.5b). On the right dry bank between *A. mearnsii* trees, scattered exotic, spreading tangled thorny bramble (*Rubus fruticosus*) were found throughout the riparian corridor. On the left bank, typical mountain fynbos was found, including *B. stellatifolium*, *M. angustifolia* and *Searsia angustifolia*. The left bank was dominated by bramble in the riparian area. *Acacia mearnsii* stands were only found further downstream close to the starting point of the wetland, with trees being of similar height. Some algae were present in the middle of the reach and on the edges of the river bank.

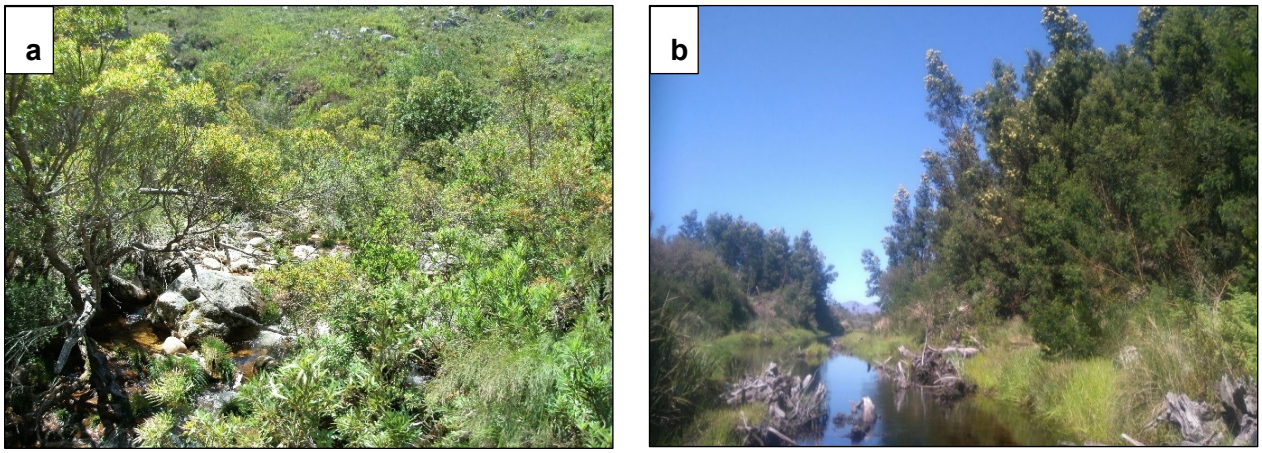

Figure 2.5: near pristine (**a**) and invaded site (**b**) at the Du Toit's River. Photographs were taken in the summer month of December 2016.

2.3. Methodology

2.3.1. Leaf litterfall traps

Litter traps were placed under both native and alien tree canopies in the near pristine and invaded sites of the Wit and Du Toit's Rivers. At the invaded sites, ten traps were placed in a random order under a closed canopy *of A. mearnsii* trees. Here a closed canopy was to have at least 50% cover or more (Figure 2.6a). Five traps were place for each of the two native tree species, *B. stellatifolium* and *M. angustifolia.* Traps could not be placed randomly as the vegetation cover of the native species was not as dense as that of the invaded species (Figure 2.6b).

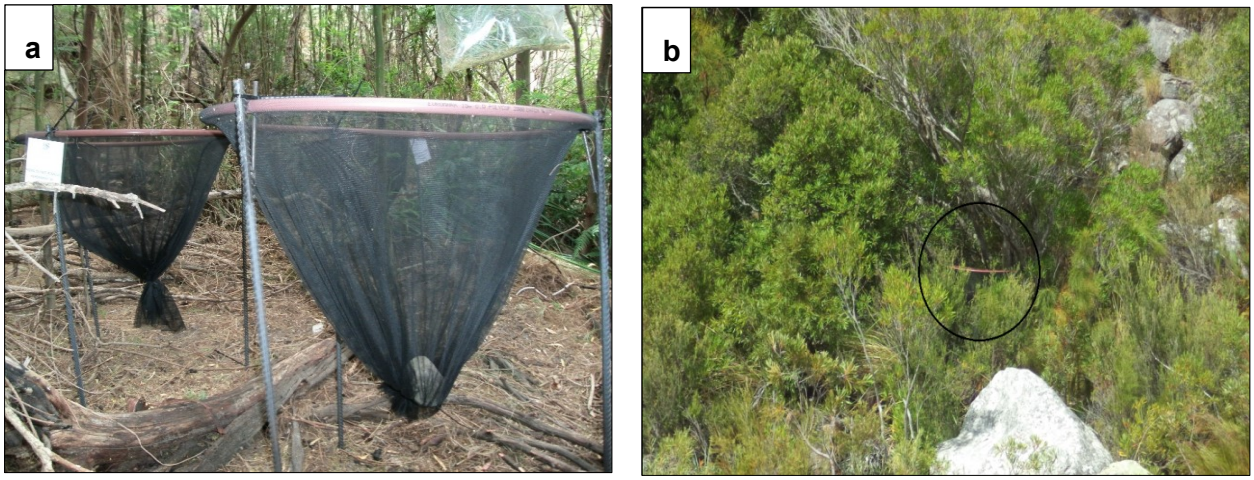

Figure 2.6: Leaf litter traps in (**a**) dense *A. mearnsii* stands in the Du Toit's River invaded sites and (**b**) a litter trap under a *M. angustifolia* tree at the Wit River, near pristine site.

Litterfall and other inputs were collected in a fine mesh (0.5 mm) net that was suspended at a top height between 1.0-1.3 m with a known catching area of (1.149 m^2). The traps were attached to three steel rebar rods and nets were 0.5 m deep to prevent litter from blowing out of

the traps and not fixed close to the ground to ensure ample water drainage (Figure 2.6a). The opening area of the traps was horizontal to the ground. The top height ensured that there was clearance from the ground on the up-slope side, whilst still allowing capture of leaves from the tree canopy. We followed the method of Staelens et al., (2003) for constructing litterfall traps.

In the laboratory large leaves were handpicked (mostly *B. stellatifolium*), while smaller leaves, especially phyllodes of *A. mearnsii* was separated by sieving through a 250 µm sieve which allowed retention of mostly leaf litter material. Hereafter samples were oven-dried for at least 48 h at 60°C until a constant weight was achieved. A top loading analytical balance, accurate to 0.1 g, was used to weigh leaf material. Samples were collected monthly from January 2016 to December 2016. Mean monthly values, expressed in g m^{-2} were calculated for each trap per site. Leaf samples of each month (12 months) were weighed out to approximately 5 g for each species, ground in a rotary mill and sieved through a 150 µm sieve to a homogeneous powder. The homogeneous powder of the different species was weighed between 0.5 - 0.8 mg in tin foil cups on an ultramicrobalance (Mettler Toledo XP6) as prescribed by laboratory standards from the Geology Department at the Stellenbosch University. Thereafter seasonal N and C concentrations (reported in milligram per gram dry mass) were determined and expressed as elemental composition using an Elementar Vario EL Cube, hosted within the ICP-MS and XRF Unit of the Central Analytical Facilities. This allowed the calculation of the C:N ratio.

2.3.2. Isotope analysis ($\delta^{15}N$ used as indicator for N cycling)

A ^{15}N natural abundance technique was used as an indicator of the openness of the N cycle in the different plant species (Shearer and Kohl, 1986; Fry, 2006). Foliar material was sampled from *A. mearnsii* trees and nearby non–fixing reference plants, which is consisted of *B. stellatifolium* and *M. angustifolia* growing within a 5 m radius of *A. mearnsii* (Jacobs et al., 2006). Foliar samples were collected in mid-autumn (early April 2006) for $\delta^{15}N$ analysis to discriminate whether the N–fixing *A. mearnsii* and nearby non–fixing reference plants were utilizing soil nitrogen (i.e., nitrate and ammonium). Therefore, thirty trees were selected for sampling from each species. The selection of samples of leaves was specifically from the canopy of the tree, and only leaves without noticeable signs of disease were collected. Samples were labelled and placed in brown bags in the field and returned to the laboratory.

Foliar samples were dried at 70°C for 3 days, then ground in a rotary mill, and weighed to 0.5 - 0.8 mg as prescribed by laboratory standards using an ultramicrobalance (Mettler Toledo XP6) and placed into tin foil cups. The tin foil samples were prepared for C and N elemental compositions and isotope analysis using a Flash HT Plus elemental analyser attached to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface. Carbon and nitrogen

isotope values were rectified against an in-house standard (Merck Gel) and a Urea Working Standard (IVA Analysentechnik e.K., Meerbusch, Germany). Blank and laboratory standard samples were run after every 12 unidentified samples. Sample analysis were carried out at NRF iThemba laboratories, Johannesburg. Stable isotopic data are reported in standard delta notation ($\delta^{15}N$, relative to atmospheric N_2 reference standard).The standard delta notation ($\delta^{15}N$) was used as an indicator of N cycling between species. The difference between the ratios of atoms of ^{15}N:^{14}N in the plant samples (Rsample) and that in a standard such as air (Rstandard) is expressed in terms of $\delta^{15}N$ in parts per 1000: Equation. 2.1.

$$\delta(15N) = \frac{\text{Rsample-Rstandard}}{\text{Rstandard}} \text{ X } 1000 \qquad \text{(Eq.2.1)}$$

2.3.3. Resorption efficiencies (Retranslocation of nutrients in *A. mearnsii* and native plant species)

The resorption efficiency of nutrient's (especially N and P) was determined from senesced leaves, which gives us the ability to understand resorbtion of nutrients and can be seen as a vital component of an adaptive mechanism to conserve nutrients in the riparian zones in the two perennial rivers (Zhang et al., 2014. Samples of mature green leaves and senesced leaves were collected from the native species in the near pristine site and *A. mearnsii* in the invaded site at each river to estimate nutrient cycling. Five trees were selected for sampling from each species in December (early summer) 2016. Senesced leaves are those leaves, which are different in colour (often red or yellow) and can be removed from the twig by a gentle flick of the leaf (Norby et al., 2000; Wright and Westoby, 2003).

Leaves (senesced) were collected directy from the plant, rather than from the leaf litter traps. It was done to avoid the effect of leaching and decomposition of leaf litter nutrients. Leaf material at the collection time was collected randomly from different points at canopy and pooled by species for nutrient analysis. In the laboratory they were oven-dried for 72 h at 60°C to determine dry mass (g). Samples was finely ground to a homogenous powder using a 'Hammer Mill' with a sieve size of 250 µm and stored in plastic micro–centrifuge tubes. Finally, samples were transported to a commercial laboratory, Bemlab in Somerset West in the Western Cape of South Africa. A Leco instrument (FP-2000 model) and Combustion Method was used to analyse N and ICP instrument to analyse P content. The content were calculated per unit mass (reported in milligram per gram dry mass). Resorption efficiency and proficiency were calculated per individual, providing insight into both the intraspecific and interspecific variation in resorption

constraints. The percentage difference in N and P between green and senesced leaves were calculated using the equation: Equation 2.2.

$$\text{resorption efficiency (RE)} = \frac{[\text{nutrient}]\text{green}-[\text{nutrient}]\text{sen})}{[\text{nutrient}]\text{green}} \times 100 \qquad \text{(Eq.2.2)}$$

Where the N or P concentration in mature green leaves is $[\text{nutrient}]_{green}$ and $[\text{nutrient}]_{sen}$ is the senesced leaf N or P concentrations (Aerts, 1996). Leaf senescence was sometimes difficult to judge, especially for the species of *B. stellatifolium*. Some of the senesced leaves collected at times have not completely undergone the process at time of collection. Therefore we chose to use the 0.10 quartile values for $[\text{nutrient}]_{sen}$ (calculated as the lower 10^{th} percentile of senesced leaf N or P concentrations) under the presumption that they are leaves that had undergone the process of complete senescence or near–complete senescence. The resorption proficiency was determined using $[\text{nutrient}]_{sen}$ as defined in the equation.

2.3.4. Leaf litter decomposition and macroinvertebrates sampling

Leaves of *A. mearnsii* and two native species, *B. stellatifolium* and *M. angustifolia* were collected from single trees in December 2015 and November 2016 just before abscission or shortly after they had fallen (Norbly et al., 2000). All leaf material were oven dried for 24 h at 50 °C. To test for differences in decomposition and macroinvertebrates between leaf types, and invasion status (NP or IV) leaf bags were imbedded at both rivers (Wit and Du Toit's River at the different invasion sites) from 4 November 2016 to 6 February 2017 (incubation periods of 14, 28, 48, 64, 80 and 102 days). Leaf bags were made out of nylon fine mesh (0.5 mm) for the decomposition rate experiment to exclude macroinvertebrates (Webster and Benfield, 1986). The decomposition experiment bags was design so that the decomposition of leafs were driven by in stream characterstics (bacterial/fungal) and leaf composition (Graça et al., 2001). Macroinvertebrates bags were made out of fine – nylon coarse mesh (0.5 mm with a 2 mm screening window) to allow access to macroinvertebrate herbivores (Figure 2.7). The surface area of leaf bags for both experiments was 0.04 m^2. A total of 5.0 g of *A. mearnsii* leaves were placed into litter bags, and for the native species litter was weighed out to 2.5 g for each species, mixed and placed into litter bags, hereafter called 'Fynbos species'. A total of 384 (192 per river system) leaf bags were fabricated for the decomposition and macroinvertebrate experiments.

In order to test the home field advantage (HFA) hypothesis that leaf litter tends to decompose faster in its home environment and macroinvertebrates in the same environment favours the plant material above them (Austin et al., 2014), fynbos species litter (HFA) and *A. mearnsii* (HFA) decomposition bags were placed in the native reach (i.e. near pristine = fynbos species (HFA); foreign litter which was the *A. mearnsii* litter bags) and in the invaded site. At

every incubation date, eight bags per invasion status (NP or IV) were retrieved which consisted of the HFA leaf bags and foreign leaf bags.

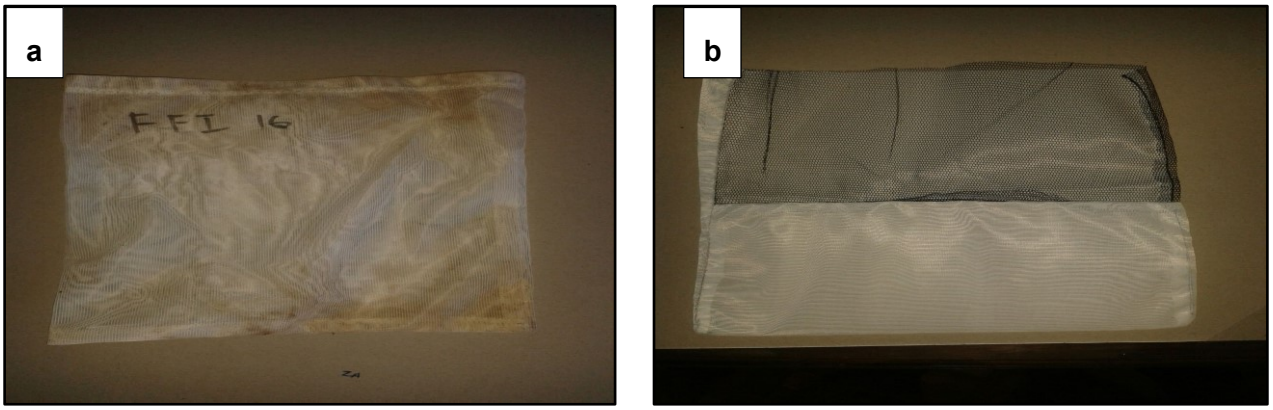

Figure 2.7: Representation of leaf bags made out of (**a**) nylon fine mesh, 0.5 mm (exclude macroinvertebrates) and leaf bags made out of half (**b**) nylon fine and coarse mesh, 0.5 mm with a 2 mm screening window (included macroinvertebrates).

The same procedure was followed for the macroinvertebrate experiment. At the initial placement of bags all 384 leaf bags (nylon fine mesh, 0.5 mm which exclude macroinvertebrates and fine-coarse mesh, screening window which included macroinvertebrates were placed in the stream. In total 32 bags were retrieved per river system at every incubation period, labelled with unique codes (Table 2.1). Four leaf bags (of both experiments) were tied to a steel rod with gutter nails at each reach. The steel rod was used an anchor against any obstruction that might flow downstream due to strong flow variability (Webster and Waide, 1982).

Table 2.1: Retrieval schedule for leaf bags of decomposition and macroinvertebrate experiments, in near pristine and invaded reaches at the Wit (**a**) and Du Toit's River (**b**) sites. HFA = the species in its home environment.

Study site:	**Wit River (a)**	**Du Toit's River (b)**
Retrieval schedule	Decomposition	
Near pristine reach:		
Fynbos species (HFA)	4 leaf bags	4 leaf bags
A. mearnsii	4 leaf bags	4 leaf bags
Invaded reach:		
***A. mearnsii* (HFA)**	4 leaf bags	4 leaf bags
Fynbos species	4 leaf bags	4 leaf bags
	macroinvertebrate	
Near pristine reach:		
Fynbos species (HFA)	4 leaf bags	4 leaf bags
A. mearnsii	4 leaf bags	4 leaf bags
Invaded reach:		
***A. mearnsii* (HFA)**	4 leaf bags	4 leaf bags
Fynbos species	4 leaf bags	4 leaf bags

The Hierarchical Framework for Stream Habitat Classification by Frissel et al., (1986) was followed to place both experiments leaf bags. Microhabitat subsystems are systems defined by Frissel et al., (1986) as patches within pool/riffle systems which have similar environmental conditions (temperature, discharge, substrate characterization). Decomposition and macroinvertebrate leaf bags were placed in sections where microhabitat subsystems were detected within pool systems (Frissell et al., 1986). Mediterranean-climate streams are categorised by successive floods and droughts that are variable through seasons due to high annual and inter-annual discharge (Gasith and Resh, 1999). Pools are seen as habitats which hold inputs of leaf litter and other debris which accumulates and creates new microhabitats through different seasons (Gasith and Resh, 1999; Bonada et al., 2007). Macroinvertebrate and decomposition bags were placed in pool habitats as these sections of a stream are generally less affected by flow variability and major changes in synergistic factors (water temperature, pH and conductivity, Grab, 2014).

Leaf bags for decomposition and macroinvertebrate experiments were collected in zip-lock bags and transported to the laboratory. The bags were disassembled, where the remaining leaf material in each bag was carefully washed off and sieved (250 µm) to remove debris and invertebrates. Material at each incubation day over the study period of decomposition were placed in paper bags with labels oven-dried for 24 h at 50 °C to attain a constant dry mass (g). Sometimes mineral deposits were not washed off the leaves resulting in incorrect final dry mass. This was solved by converting dry mass of each leaf pack to ash-free dry mass (AFDM). The dry mass of each bag was placed into a muffle furnace at 550 °C as organic matter combust at these temperatures (Webster and Benfield, 1986). The mineral ash was then substracted from the dry mass initially recorded which gives the ash-free dry mass or AFDM of each leaf bag. The AFDM represents the percentage mass loss (%) at each inbucation day over the period. To determine ADFM the following equation must be used (Equation 2.3).

% AFDM reamining = Final AFDM/Initial AFDM X 100 (Eq.2.3)

In order to calculate the decomposition rate over time, the expotential model of (Olson, 1963) was used. Where the percentage mass loss at the start of the experiment was (W_0) and and percentage mass loss at time t was (W_t). The constant k is expressed in unit (day^{-1}) and was calculated for each litter bag at each incubation day (Equation 2.4).

$$W_t = W_0 e^{-kt} \quad \text{(Eq.2.4)}$$

The invertebrates retained in the experiment were preserved in 90% ethanol in 50 ml vials for later identification. Macroinvertebrates were identified to genus level with an Olympus SZ compound microscope (Model SZ2-ILST) and using the WRC freshwater invertebrate guides (Day et al., 2002b; de Moor et al., 2002b; de Moor et al., 2003b; Stals and de Moor, 2007). The identification of genera belonging to the functional feeding groups (FFG's) of scraper, deposit feeder and predators were computed according to their FFG's. (2) The genera of these FFG's were then summed within families. (3) Functional Feeding Groups (FFG's) were assigned using Schael, (2005) and the modified version by Merrit and Cummins, (1984). Finally, functional feeding group abundance was standardized to 1 m^2 according to the available surface area of the mesh bag before calculating abundances of the functional feeding groups. The genera identified, their abundances and which functional feeding groups they fall into are reported.

2.3.5. Environmental parameters

Water temperature (°C) at each invasion status of each river was recorded every 30 minutes through the experiment (November 2016 – February 2017) with a HOBO U20L Water Level Logger (Onset Computer Corporation Inc., Pocasset, MA, USA). Instantaneous discharge measurements were made at every sampling event with a hand-held Marsh-McBirney Model 2000 flow meter from Southern Waters Consultancy. Similarly, stream water pH and electrical conductivity (EC) were recorded in the field throughout the experiment using a Hanna Multiparameter Waterproof Meter (Model - HI9829) from the University of the Western Cape, Earth Sciences Department.

Chapter 3

Nitrogen Inputs from invasive *Acacia mearnsii* into riparian zones in the Fynbos biome (CFR), South Africa

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3.1. Abstract

Invasive Australian *Acacia* tree species are classified as among the most harmful of invasive species to the fynbos vegetation. They are also most prevalent in Cape Floristic Region (CFR), a global biodiversity hotspot. Black wattles (*Acacia mearnsii*) are leguminous nitrogen fixer and make significant contributions to available nitrogen in their new habitats, which are nutrient poor. However, how much nutrients and the timing of input into riparian zones and adjacent aquatic environments are not known. In this study, I assessed patterns of leaf litterfall, N cycling and nutrient resorption (N and P) capacities of *A. mearnsii* and two native co-occurring species in two perennial streams in the Fynbos biome of the CFR. Annual leaf litterfall of *A. mearnsii* in riparian zones was seven to ten times greater in *A. mearnsii* species (on average 297.49 g m^{-2}) compared to native vegetation (on average 34.44 g m^{-2}), with two peaks in litterfall, one in mid-autumn, and the other in mid-summer at both perennial streams. In contrast, the native vegetation only drop leaves once a year in the early summer month of December. *Acacia mearnsii* also retained higher N concentrations with a mean value of 24.82 mg g^{-1} in leaf litter than the native species throughout the year, which ranged from 3.23 to 8.63 mg g^{-1} between seasons. The $\delta^{15}N/\delta^{14}N$ isotope ratio showed positive $\delta^{15}N$ signatures in *A. mearnsii* suggesting that nitrogen cycling may proceed faster within stands of *A. mearnsii* and that the N cycle here may be more open. The co-occurring native species were more efficient in the resorption of nutrients (N and P) which was on par with global averages of 56%. In contrast, *A. mearnsii* was inefficient in the resorption of N with an average of 21.86 % resorbed, but was more efficient in resorbing P (± 50%). It is possible that the *A. mearnsii* stands rely on the return in N rich leaf litter under soils and through specialized root systems and mycorrhizal symbionts assisting reuse of nutrients. The results highlight the differences in nutritional economy between native riparian species and the invasive *A. mearnsii* and provide further insight to the success of leguminous woody tree species in nutrient poor Mediterranean ecosystems.

KEYWORDS: Australian Acacia; fynbos vegetation; Cape Floristic Region; nutrient poor; leaf litterfall; N cycling; nutrient resorption; root systems; mycorrhizal symbionts; nutritional economy

3.2. Introduction

The conservation of biodiversity is a major challenge for ecosystem management. After habitat loss the second biggest global threat of biodiversity is invasive alien plant species (IAP's) (D'Antonio and Meyerson, 2002; Richardson and van Wilgen, 2004). Riverine systems, including riparian habitats are extremely vulnerable to invasion and the spread of non-native species due to its linear nature and disturbance regime that favours invasive plants (Hood and Naiman, 2000; Kuglerova et al., 2017). The successful invasion of exotic species in riparian vegetation communities can be harmful to riparian biodiversity and ecosystem process such as nutrient cycling, soil microbial community and soil nitrogen levels (Richardson et al., 2007; Kuglerova et al., 2017). IAP's have the ability to to establish themselves in non-native environments which is dependent on their ability to outgrow and outcompete native riparian vegetation for light, water and nutrients, which are essential to the species survival and growth (Vitousek et al., 1997b; Morris et al., 2011). Australian *Acacia* spp., which consist of 1012 species in the subgenus Phyllodineae, native to Australia (Richardson et al., 2011) are one of most pervasive alien plant groups in the world (Richardson and van Wilgen, 2004; Lowe et al., 2008;). These Australian *Acacia* spp. compete successfully in disturbed environments (for instance, riparian corridors and post-fire environments; Richardson et al., 2007) and particularly in landscapes that are limited in nutrient such as the Mediterranean-type ecosystems like the dunes in Portugal and in the Fynbos biome of the CFR, South Africa (Witkowski, 1991a; Brown et al., 2004; Marchante et al., 2008).

In the Fynbos biome, the most common Invasive Australian *Acacia* tree species. (*Acacia mearnsii, A. longifolia, A. saligna*) that invade the biome's fynbos plant communities are able to fix nitrogen from the atmosphere, forming root nodules where symbiotic nitrogen-fixing bacteria make nitrogen available for the plant to absorb (Drake, 2011; Morris et al., 2011). These woody invaders alter nitrogen, carbon, phosphorus and water cycles (Yelenik et al., 2004; Morris et al., 2011; Naude, 2012). Previous studies (e.g. Milton, 1981; Witkowski, 1991a; Yelenik et al., 2004, 2007; Naude, 2012) has shown how leguminous IAPs increase available nitrogen in the soil through increased inputs of nutrient rich leaf litter. As result, high N inputs can be anticipated in the long run and may help predict ecosystem disruption especially through elemental cycling by N_2-fixing plants (Stock et al., 1995; Vitousek et al., 1997b). The cycling of nuntrients, notably nitrogen in natural vegetation is slow as fynbos biome plant species consist largely of sclerophyllous shrubs, a characteristic of plant assemblies in nutrient poor landscapes as the Mediterranean climate regions (Stock and Allsopp, 1992). Fynbos plants grow slowly and have high nutrient cycling prior to leaf abscission (Stock and Allsopp, 1992). Many studies found that natural vegetation have litter with low levels of N concentrations, high carbon-to-nitrogen ratio, slower rates of decomposition than the N_2-fixing plants to which they are normally compared to

(Witkowski, 1991a; Liao et al., 2008). Native N_2-fixing plants exist in the fynbos, however they are short lived and distributed erratically in the post-fire plant species community. In addition, these early colonizing species do not deposit enough of their N rich leaf-litter to have an impact on soil N dynamic status, thus making them a lesser influence on ecosystem nutrient cycling (Yelenik et al., 2004). Stands of trees of *Acacia* spp. are in most instances three times taller than that of the fynbos species in the surrounding area and contain a larger quantity of biomass (including foliar biomass) than their native counterparts (Milton 1981, Yelenik et al., 2004, 2007).

Various research studies point out that *A. mearnsii* influences ecosystem functioning and native diversity (Caldeira et al., 2002; Moyo and Fatunbi, 2010; Tye and Drake, 2011; Van der Colff et al., 2017) and uses more water than native species, which reduces stream flow (Le Maitre et al., 2002). *Acacia mearnsii* is a nitrogen fixer, which has the ability to nodulate under water and nutrient limited conditions (Sprent, 2009; Crous, 2010; Rodriguez-Echevarria, 2011) due to specialized mechanisms such as extensive root systems (extensive and deep), symbiotic N_2-fixation through the bacterium *Rhizobium* spp. and nutrient conservation strategies (Morris et al., 2011; Van der Colff et al., 2017). The ability of *A. mearnsii* to maintain high growth rates permits them to overgrow and outcompete native species (Milton, 1981; Witkowski 1991a). *Acacia mearnsii* consists mostly large, dense, thick stands of evergreen shrubs between 1 – 20 m tall, which differs from most native species (riparian community plants), thus allowing *A. mearnsii* to occupy previously unexploited niches (Rascher et al., 2011). The dense stands and rapid growth rates of *A. mearnsii* consequently leads to higher above ground biomass than the native species (Moyo and Fatunbi, 2010; Rascher et al., 2011). *Acacia mearnsii* has unique features such as large growth rates which has leaf litter that is rich in nutrients and the ability to fix biological nitrogen and lastly the resorption of these crucial nutrients for better growth and survival (Morris et al., 2011; Tye, 2013; Van der Colff et al 2017). As a consequent of the unique trait features, the species alters native plant communities more severely than through direct rivalry. Therefore, invasive *Acacia* spp., especially *A. mearnsii* are considered a successful and persistent nitrogen fixing species in any terrestrial ecosystem (Lawrie, 1981; Vitousek et al., 1997a; Yelenik et al., 2004; Rodrı´guez- Echeverrı´a et al., 2011).

However, the extent to which *A. mearnsii* adds and recycles N within riparian and aquatic ecosystems (litterfall, N concentrations, and resorption of nutrients) is not well understood in the nutrient impoverished ecosystems of the Fynbos biome. To examine this, we quantified the difference in litter quantity and quality between the woody *A. mearnsii* compared to two native keystone species*; Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae) of the Mediterranean-type riparian zones in the Fynbos biome of the CFR. The knowledge gained around this study will give a better insight of N contribution to riparian zones and adjacent aquatic environments. This will in due course improve our understanding of the

nutrient economy of *A. mearnsii* in the nutrient poor fynbos riparian zones in the Fynbos biome of South Africa.

3.3. Site descriptions, Methods and Materials

3.3.1. Study areas in the Fynbos biome of the CFR

This study was conducted in the Western Cape in the mountain streams and mountain transitional zones of the Mediterranean-type Fynbos biome (Reinecke et al., 2013) in the Breede Water Management Area (WMA). Two perennial rivers were chosen for the study the Wit River in Bainskloof Pass and the Du Toit's River in the Franschhoek Pass which is cut short by the Theewaterskloof Reservoir (Tharme and Brown, 2004). Along each river, sites within two invasion statuses were identified: (1) near pristine site (NP), and (2) invaded site (IV). Near pristine sites had to be characterised by typical mountain fynbos, including individuals of *B. stellatifolium*, *M. angustifolia*, *S. angustifolia* and short indigenous trees, bushes and sedges with no alien invasive trees present. Invaded sites had to include dominant *A. mearnsii*, while some *A. longifolia* may be present, with an IAP cover of over 50% at the sites. Sites were arranged from up - to downstream with near pristine sites being upstream and invaded sites being downstream. In addition, no commercial or agricultural areas had to be in close vacinity of the study sites.

The CFR is characterized by Mediterranean-type climate of summers which is dry and winters that are wet. After the first heavy rains, mountain streams and rivers in the CFR have ample surface flow in winter and in summer many streams are reduced to perennial pools and section of riffles resulting in periodic water shortages (Goldblatt and Manning, 2000; Tharme, 2010). In the Fynbos biome vegetation distribution in upper catchments are typically characterised by sclerophyllous fynbos and sandstone soils which are very acidic and nutrient poor (Day and King, 1995; Samways et al., 2011). Sampling started in January 2016 and lasted until December 2016.

3.3.2. Leaf litterfall traps and seasonal C, N concentrations

Litter traps were placed under both native and alien tree canopies in the near pristine and invaded sites of the Wit and Du Toit's Rivers. At the invaded sites, ten traps were placed in a random order under a closed canopy of *A. mearnsii* trees. Here a closed canopy was to have at least 50% cover or more. The method of Staelens et al., (2003) for constructing litterfall traps was followed. Five traps were place for each of the two native tree species, *B. stellatifolium* and *M. angustifolia*. Traps could not be placed randomly as the vegetation cover of the native species was not as dense as that of the invaded species. In some cases, especially in near pristine sites trap fixtures were used, these fixtures such as rope and tie straps were used to fix the traps sturdily on the ground as the study was conducted in the mountainous riparian plots (Staelens et al., 2003). Litterfall and other inputs were collected in a fine mesh (0.5 mm) net that was suspended at a top height between 1.0 - 1.3 m with a known catching area of (1.149 m^2). Each trap was attached to three steel rebar rods and equally spaced out to ensure adequate water drainage and a depth of 0.5 m prevent litter from blowing out of the traps.

In the laboratory large leaves were handpicked (mostly *B. stellatifolium*), while smaller leaves, especially phyllodes of *A. mearnsii* was separated by sieving through a 250 µm sieve which allowed retention of mostly leaf litter material. Hereafter samples were oven-dried for at least 48 h at 60°C until a constant weight was achieved. A top loading analytical balance, accurate to 0.1 g, was used to weigh leaf material. Samples were collected monthly from January 2016 to December 2016. Mean monthly values, expressed in g m^{-2} were calculated for each trap per site. Leaf samples of each month (12 months) were weighed out to approximately 5 g for each species (invasive and native species), ground in a rotary mill and sieved through a 150 µm sieve to a homogeneous powder. The homogeneous powder of the different species was weighed between 0.5 - 0.8 mg in tin foil cups on a ultramicrobalance (Mettler Toledo XP6) as prescribed by laboratory standards by the the Geology Department at the Stellenbosch University. Thereafter, seasonal N and C concentrations (reported in milligram per gram dry mass) were determined and expressed as elemental composition using an Elementar Vario EL Cube, hosted within the ICP-MS and XRF Unit of the Central Analytical Facilities. This allowed the calculation of the C:N ratio.

3.3.3. Isotope analysis ($\delta^{15}N$ used as indicator for N cycling)

A ^{15}N natural abundance technique was used as an indicator of the openness of the N cycle in the different plant species (Shearer and Kohl 1986; Fry, 2006). Foliar material was sampled from *A. mearnsii* trees and nearby non–fixing reference plants, which consisted of *B. stellatifolium* and *M. angustifolia* growing within a 5 m radius of *A. mearnsii*. Foliar samples were collected in mid-autumn (early April 2006) for $\delta^{15}N$ analysis to discriminate whether the N_2–fixing *A. mearnsii* and

nearby non–fixing reference plants were utilizing soil nitrogen (i.e., nitrate and ammonium). Therefore, thirty trees were selected for sampling from each species. The selection of samples of leaves was specifically from the canopy of the tree, and only leaves without noticeable signs of disease were collected. Samples were labelled in the field, and placed in brown paper bags and returned to the laboratory.

Foliar samples were dried at 70°C for 3 days, then ground in a rotary mill, and weighed to 0.5 - 0.8 mg as prescribed by laboratory standards using an ultramicrobalance (Mettler Toledo XP6) and placed into tin foil cups. The tin foil samples were prepared for C and N elemental compositions and isotope analysis using a Flash HT Plus elemental analyser attached to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface. Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel) and a Urea Working Standard (IVA Analysentechnik e.K., Meerbusch, Germany). Blank and laboratory standard samples were run after every 12 unidentified samples. Sample analysis was carried out at NRF iThemba laboratories, Johannesburg. Stable isotopic data are reported in standard delta notation ($\delta^{15}N$, relative to atmospheric N_2 reference standard). Stable isotopic data are reported in standard delta notation ($\delta^{15}N$, relative to atmospheric N_2 reference standard).The standard delta notation ($\delta^{15}N$) was used as an indicator of N cycling between species. The dissimilarity between the ratios of atoms of ^{15}N:^{14}N in the plant samples (Rsample) and that in a standard such as air (Rstandard) is expressed in terms of $\delta^{15}N$ in parts per 1000: Equation. 3.1.

$$\delta(15N) = \frac{Rsample - Rstandard}{Rstandard} \times 1000 \qquad \text{(Eq. 3.1)}$$

3.3.4. Resorption efficiencies (Retranslocation of nutrients in *A. mearnsii* and native plant species)

The resorption efficiency of nutrient's (especially N and P) was determined from senesced leaves, which gives us the ability to understand resorbtion of nutrients and can be seen as a vital component of an adaptive mechanism to conserve nutrients in the riparian zones in the two perennial rivers (Zhang et al., 2014. Samples of mature green leaves and senesced leaves were collected from the native species in the near pristine site and A. mearnsii in the invaded site at each river to estimate nutrient cycling. Senesced leaves are those leaves, which are a different colour often red or yellow and can be removed from the twig by a tender flick of the leaf (Norby et al., 2000; Wright and Westoby, 2003).

Leaves (senesced) were collected directy from the plant, rather than from the leaf litter traps. It was done to avoid the effect of leaching and decomposition of leaf litter nutrients. Leaf material at the collection time was collected randomly from different points at canopy and pooled by species for nutrient analysis. In the laboratory they were oven-dried for 72 h at 60°C to determine dry mass (g). Samples was finely ground to a homogenous powder using a 'Hammer Mill' with a sieve size of 250 µm and stored in plastic micro–centrifuge tubes. Finally, samples were transported to a commercial laboratory, Bemlab in Somerset West in the Western Cape of South Africa for N and P analysis using a Leco instrument (FP-2000 model) and combustion method for N and ICP instrument for N and P content were calculated per unit mass (reported in milligram per gram dry mass). Resorption efficiency and proficiency were calculated per individual, and then pooled by species for the purpose of comparison, providing insight into both the intraspecific and interspecific variation in resorption constraints. The percentage difference in N and P between green and senesced leaves were calculated using the equation: Equation. 3.2.

$$\text{resorption efficiency (RE)} = \frac{[\text{nutrient}]\text{green} - [\text{nutrient}]\text{sen})}{[\text{nutrient}]\text{green}} \times 100 \qquad \text{(Eq. 3.2)}$$

Where the mean N or P concentration in mature green leaves is $[\text{nutrient}]_{\text{green}}$ and $[\text{nutrient}]_{\text{sen}}$ is the senesced leaf N or P concentrations (Aerts, 1996). Leaf senescence was sometimes difficult to judge, especially for the species of *B. stellatifolium*. Some of the senesced leaves collected at times have not completely undergone the process at time of collection. Therefore we chose to use the 0.10 quartile values for $[\text{nutrient}]_{\text{sen}}$ (calculated as the lower 10^{th} percentile of senesced leaf N or P concentrations) under the presumption that they are leaves that had undergone the process of complete senescence or near–complete senescence. The resorption proficiency was determined using $[\text{nutrient}]_{\text{sen}}$ as defined in the equation.

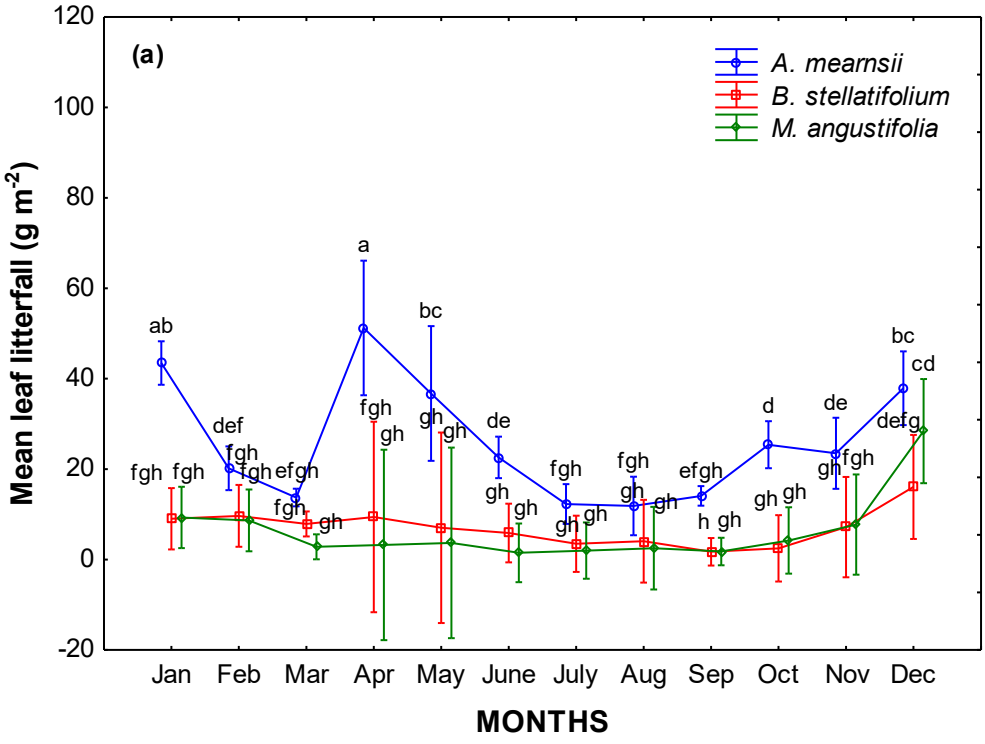
3.4. Statistical analysis

The data was analysed using the STATISTICA version 13 software package (Dell Inc., 2015). A repeated measures (two way) analysis of variance (ANOVA) was used to analyse leaf litterfall between species and months.The seasonal C and N concentrations were analyzed using a two way repeated measures (ANOVA). The Fisher LSD post-hoc test for means separations were used when significant difference were found. Foliar $\delta^{15}N$ was analyzed between *A. mearnsii*, *B. stellatifollium* and *M. angustufolia* by using a one-way (ANOVA) test followed by Kruskal-Wallis analysis for multiple comparisons (3 species) and described using the median, minimum and maximum values. Resorption efficiencies of N and P concentration in leaves were analysed by calculating the mean and standard deviation [SD] of all species.

3.5. Results

3.5.1. Monthly and seasonal leaf litterfall of *A. mearnsii* and co–occurring native species in CFR riparian zones

At the Wit River, *A. mearnsii* produced higher mean leaf litterfall rates than two co-occurring native species in months ($F_{[22,187]}$ = 2. 97, p<0.001) and in different seasons ($F_{[6.51]}$ = 2. 76, p<0.001) (Figure 3.3). This was especially evident in autumn (April) and summer (December). The highest mean leaf litterfall rate for *A. mearnsii* at the Wit River was in April (mean = 51.23 g m^{-2}) with a similar leaf litterfall rate in December with a mean of 43.47 g m^{-2} (Figure 3.1a). Conversely, the mean leaf litterfall rate for the two co-occurring native species (*B. stellatifolium* and *M. angustifolia*) remain relatively constant throughout the year with peaks of mean = 16.06 g m^{-2} and mean = 28.41 g m^{-2}, in December (Figure 3.1a). Through different seasons, *A. mearnsii* had the peak leaf litterfall occurred in autumn (mean = 33.89 g m^{-2}) compared to winter (mean = 15.58 g m^{-2}, p<0.001) and spring (mean = 20.99 g m^{-2}, p<0.001), with no significant difference between autumn and summer (mean = 33. 85 g m^{-2}, p = 0.99, Figure 3.1b). Mean seasonal leaf littefall rates for *B. stellatifolium* is relatively constant through the year (mean = 36.26 g m^{-2}). Litterfall rate of *M. angustifolia* was significantly higher in summer (mean = 15.46 g m^{-2}) compared to autumn (3.25 g m^{-2}, p<0.001), winter (1.99 g m^{-2}, p<0.001) and spring (4.57 g m^{-2}, p<0.001). Overal the mean seasonal leaf litterfall rate of *A. mearnsii* was significantly higher than co-occurring native species (p<0.001, Figure 3.1b).



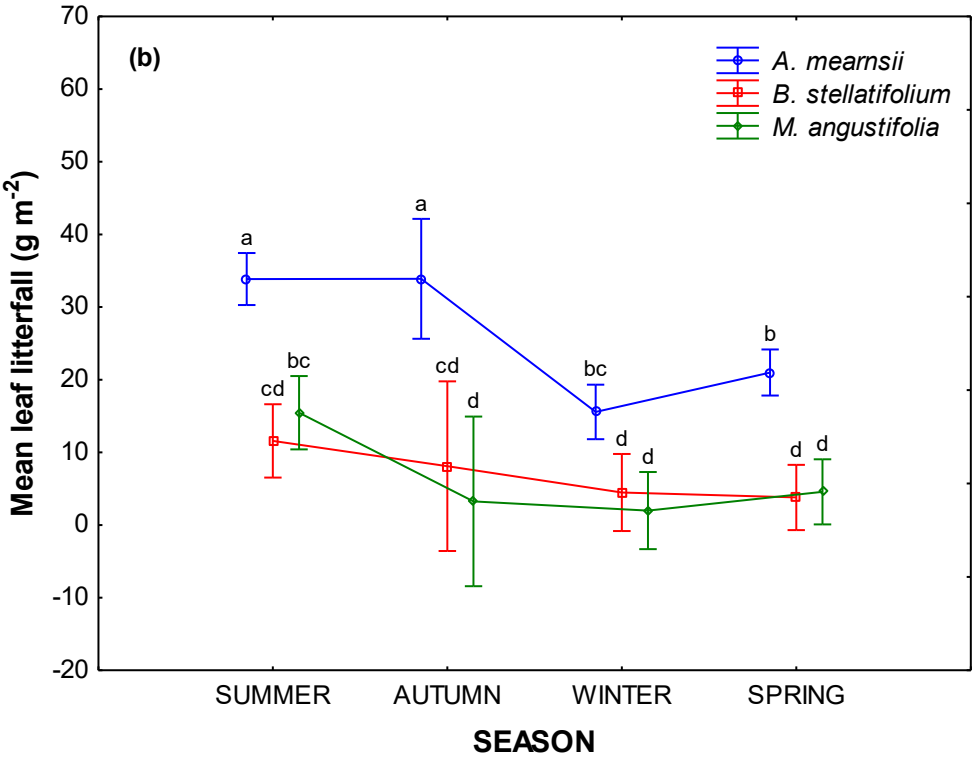


Figure 3.1: Patterns of (**a**) mean monthly and (**b**) seasonal (g m^{-2}) leaf litterfall rates for *A. mearnsii* and co–occuring native species growing in near pristine and invaded areas in riparian zones at the Wit River. Letters denote significant differences (LSD test, $p<0.05$) based on two way repeated measures ANOVA's (**a**) ($F_{[22,187]} = 2.97$, $p< 0.001$; (**b**) $F_{[6.51]} = 2.7615$, $p < 0.01$) using all the data collected over each month and different seasons.

A similar temporal trend was observed at the Du Toit's River under the dense of *A. mearnsii* stands with two peaks, one in April and one in December and for the native species only one peak in summer (December, Figure 3.2a). The highest mean monthly leaf litterfall rate for *A. mearnsii* at the Du Toit's River was in April (mean = 77.99 g m^{-2}) with December also having a relative high mean litterfall rate (mean = 44.47 g m^{-2}, Figure 3.2a). On the other hand, the mean monthly leaf litterfall rate for the two co-occurring native species (*B. stellatifolium* and *M. angustifolia*), remain relatively constant throughout the year, however there was significantly higher litterfall rates for both *B. stellatifolium* (mean = 18.56 g m^{-2}) and *M. angustifolia* (mean = 30.32 g m^{-2}, $p<0.05$, Figure 3.2a) in December. Through different seasons there were significantly higher mean seasonal leaf litterfall rates for *A. mearnsii* in autumn (mean = 49.84 g m^{-2}) relative to winter (21.88 g m^{-2}, $p<0.001$), spring (19.83, $p<0.001$) and summer (36.97, $p<0.001$, Figure 3.2b). The higest litterfall peak in *B. stellatifolium* was in summer (mean = 13 .65 g m^{-2}), which was significantly different from autumn (mean = 36.97 g m^{-2}, $p<0.01$), winter (1.96 g m^{-2}, $p<0.01$) and spring (2.35 g m^{-2}, $p<0.01$). Similar results were found *M. angustifolia* with the highest leaf litterfall peak in summer (mean = 16.92 g m^{-2}, Figure 3.2b).

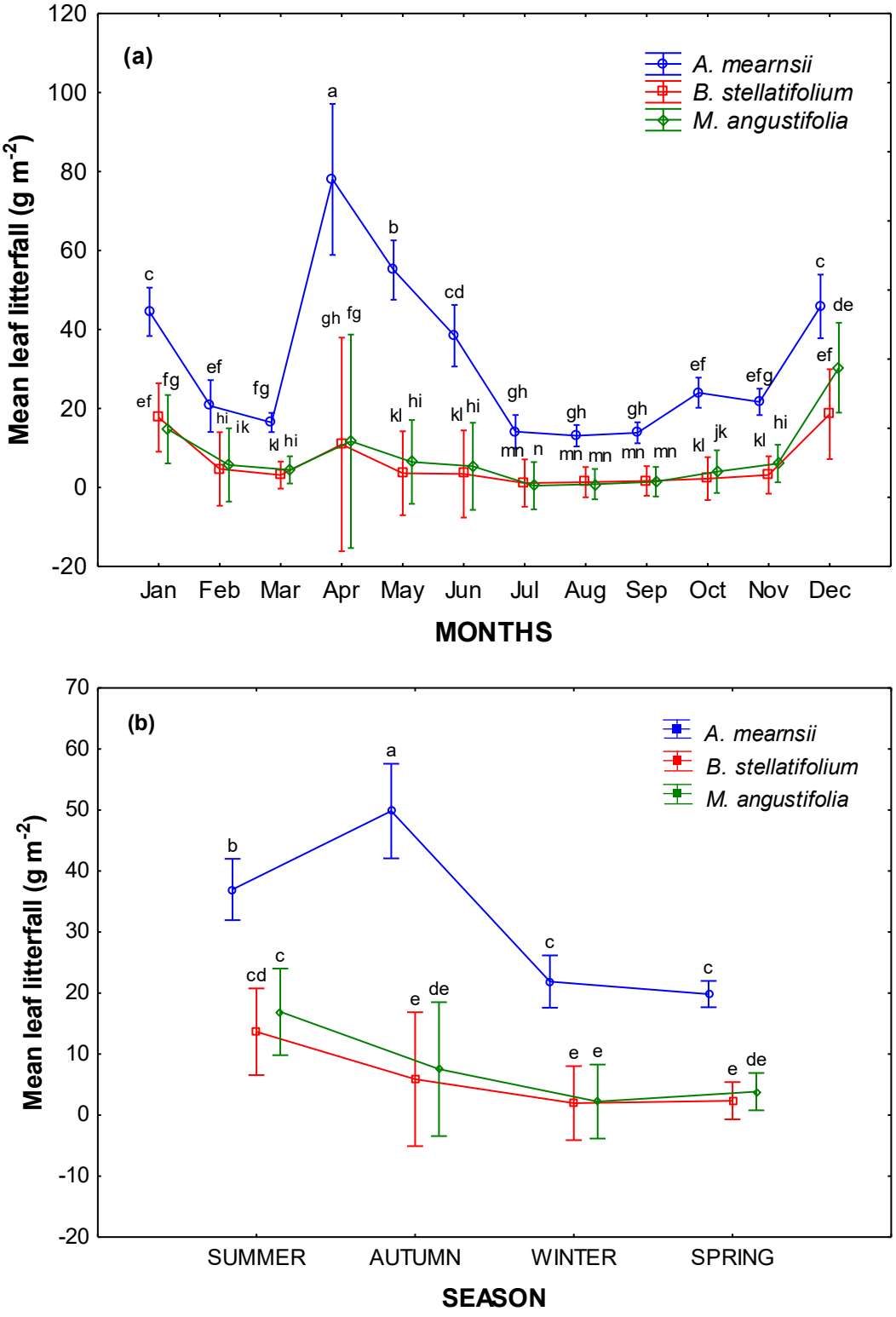


Figure 3.2: Patterns of (**a**) mean montly and (**b**) seasonal leaf litterfall rates (g m^{-2}) for *A. mearnsii* and co–occurring native species growing in near pristine and invaded areas in riparian zones at the Du Toit's River. Letters denote significant differences (LSD test, $p<0.05$) based on a two way repeated measures ANOVA's (**a**) ($F_{[22,187]} = 7.24$, $p< 0.001$; (**b**) $F_{[6.51]} = 10.11$, $p< 0.001$) using all the data collected over each month and different seasons.

On an annual basis, *A. mearnsii* had significantly more leaf litterfall (273.66 ± 37.76 g m^{-2} y^{-1}) than co–occurring native species (*B. stellatifolium* (36.26 ± 5.79 g m^{-2} y^{-1}); *M. angustifolia* (33.56 ± 11.14 g m^{-2} y^{-1}) at the riparian zone at Wit River near pristine and invaded riparian zones (Table 3.1, site a). Mean annual N return (mg N $m^{-2}y^{-1}$) was much higher over the year for *A. mearnsii* at the Wit River (Table 3.1, site a). At the Du Toit's River the same observations were made on an annual basis with *A. mearnsii* (321.32 ± 58.93 g m^{-2} y^{-1}) which had considerably higher mean annual leaf litterfall than co–occurring native species (*B. stellatifolium* (29.80 ± 9.00 g m^{-2} y^{-1}); *M. angustifolia* (38.14 ± 11.95 g m^{-2} y^{-1}) at the respective riparian zones (Table 3.1, site b). At the Du Toit's River for *A. mearnsii* was also considerably higher (8072.98 ± 2186.06 mg N $m^{-2}y^{-1}$) than the native species (Table 3.1, site b). *A. mearnsii* at the Du Toit's River generally had high N returned to soil than the Wit River invaded site, except for *B. stellatifolium* at the Wit River, which had a considerable higher N return to soil.

Table 3.1: Mean annual leaf litterfall rate (g $m^{-2}y^{-1}$) and (litterfall X N concentrations = N return to soil (mg N $m^{-2}y^{-1}$)) of *B stellatifolium* and *M. angustifolia* (N = 5) at the near pristine site and *A. mearnsii* (N = 10) in the invaded site at both Wit (**a**) and Du Toit's River (**b**). Values are represented in mean [± SD].

-	N	annual leaf litterfall rate (g $m^{-2}y^{-1}$)	annual N% return to soil (mg N $m^{-2}y^{-1}$)
Site (a): Wit River		mean ± SD	mean ± SD
Near pristine site:			
B. stellatifolium	5	36.26 ± 5.79	163.83 ± 86.51
M. angustifolia	5	33.56 ± 11.14	165.73 ± 93.51
Invaded site:			
A. mearnsii	10	273.66 ± 37.76	6896.27 ± 1554.43
Site (b): Du Toit's River			
Near pristine site:			
B. stellatifolium	5	29.80 ± 9.00	142.60 ± 96.33
M. angustifolia	5	38.14 ± 11.95	182.28 ± 102.31
Invaded site:			
A. mearnsii	10	321.32 ± 58.93	8072.98 ± 2186.06

3.5.1.1 Mean llitter C and N concentrations of *A. mearnsii* and co-occuring native species over different seasons at near pristine and invaded riparian zones

Little variation (<5 mg g^{-1}) was observed through the seasons in leaf litter N concentrations for *A. mearnsii* at the Wit River (Figure 3.3a). The highest mean leaf litter N concentrations found in *A. mearnsii* was in autumn (31.05 mg g^{-1}) with no significant differences between summer (25.58

mg g^{-1}) and winter (25 38 mg g^{-1}, p=0.93, Figure 3.3a). Mean leaf litter N concentrations in *B. stellatifolium* was highest in summer (8.36 mg g^{-1}) with no significant difference found between autumn (5.45 mg g^{-1}, p=0.08) and spring (5.28 mg g^{-1}, p=0.09). However, there was a significant difference found between the seasons of summer and winter (3.23 mg g^{-1}, p<0.001) In *M. angustifolia a* similar trend in litter N concentrations was noted with the highest mean leaf litter N concentrations being in summer (7.55 mg g^{-1}) with autumn having a matching mean of 7.34 mg g^{-1}.

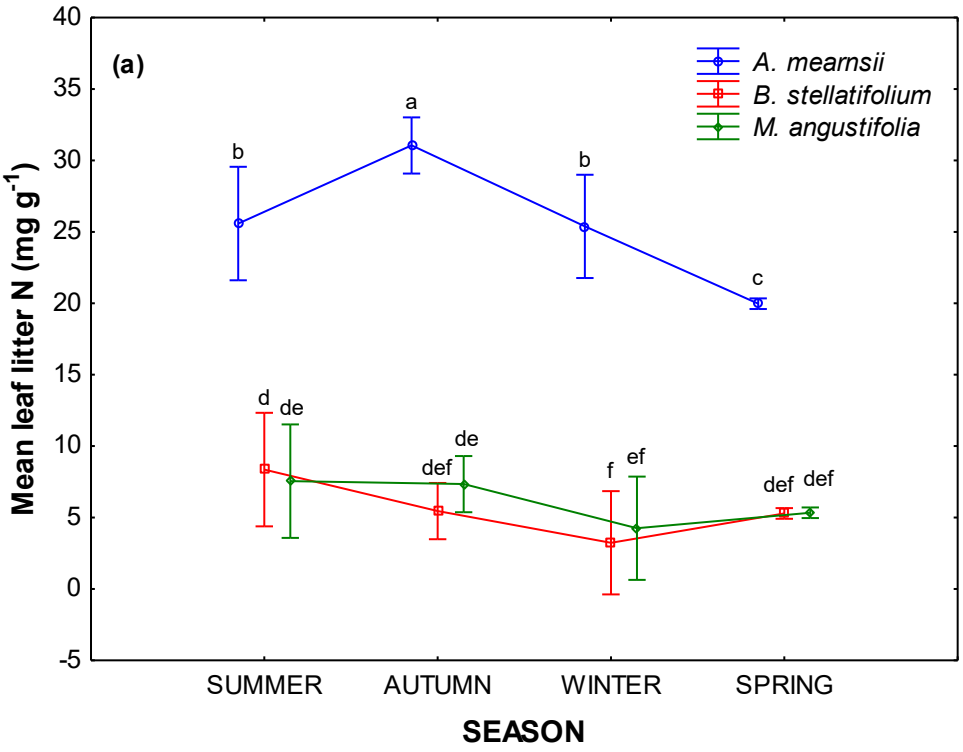


Figure 3.3: Mean seasonal leaf litter N concentrations (mg g^{-1}) for *A. mearnsii* and co–occuring native species growing in near pristine and invaded areas in riparian zones at the (**a**) Wit River. Letters denote significant differences (LSD test, p<0.05) based on a two way repeated measures ANOVA's (**a**) ($F_{[6.18]}$ =5. 47, p< 0.001) using all the data collected over different seasons.

Similar temporal variation (<5 mg g^{-1}) through seasons was noted at the Du Toit's River for *A. mearnsii* (Figure 3.4b). Mean leaf litter N concentrations are the highest in autumn (27. 97 mg g^{-1}) with no significant difference found between summer (25.44 mg g^{-1}) and winter (24.30 mg g^{-1}, p=0.27). The co-occuring native species of *B. stellatifolium* and *M. angustifolia* remain relatively constant throughout the year but with difference found between seasons. *B. stellatifolium* had the highest mean leaf litter N concentrations in summer (5.86 mg g^{-1}) with no significant difference found between autumn (5.71 mg g^{-1}, p=0.89) and spring (4.88 mg g^{-1}, p=0.35) but significant differences found between autumn and winter (3.52 mg g^{-1}, p<0.05, Figure 3.4b). Mean litter N concentration was in autumn (7.99 mg g^{-1}) for *M. angustifolia* and significant difference found between autumn and summer (5.94 mg g^{-1}, p<0.01). However,

significant differences were not found between spring and winter (4.69 mg g^{-1}, $p<0.05$, Figure 3.4b). Overall, *A. mearnsii* was significantly higher in mean leaf leaf litter N concentrations through seasons at both Wit River ($p>0.001$) and Du Toit's River ($p>0.01$).

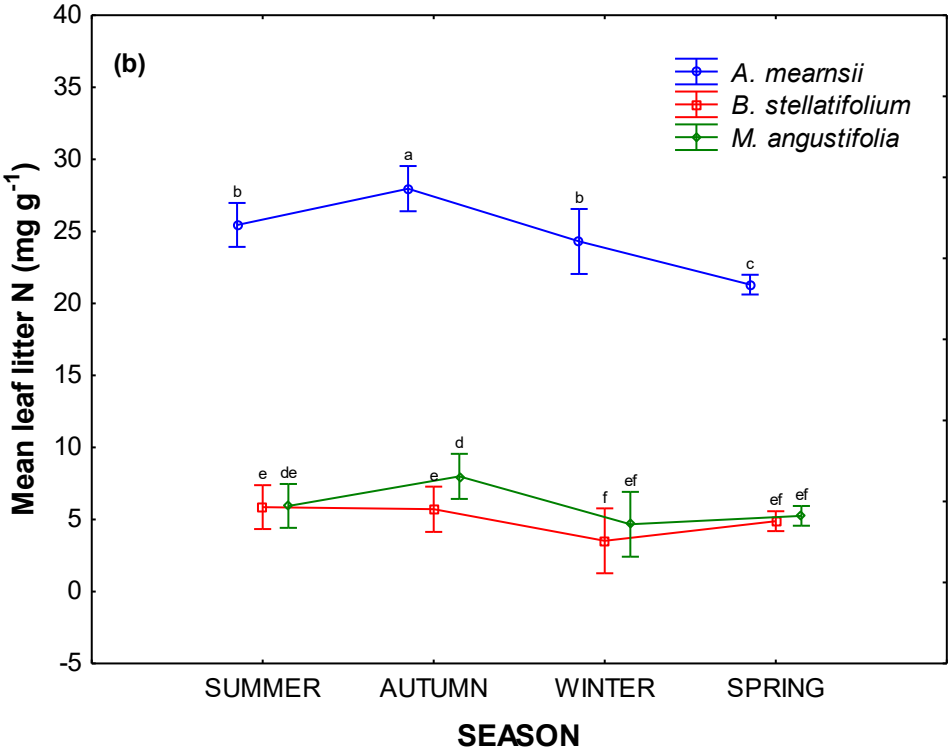


Figure 3.4: Mean seasonal leaf litter N concentrations (mg g^{-1}) for *A. mearnsii* and co–occuring native species growing in near pristine and invaded areas in riparian zones at the (**b**) Du Toit's River. Letters denote significant differences (LSD test, $p<0.05$) based on a two way repeated measures ANOVA's (**b**) ($F_{[6.18]} = 3.46$, $p< 0.01$) using all the data collected over different seasons.

C:N ratios in *A. mearnsii* are relatively constant from summer until spring at the Wit River with spring being the highest in leaf litter C:N ratios (mean = 29.21, Figure 3.5a), however no significant ($p<0.05$) differences were apparent in seasons. In the co-occuring native species, *B. stellatifolium* had the highest mean litter C:N ratio in winter (mean = 69.27) with a similar ratio found in spring (mean = 68.90) which was not significant ($p = 0.97$) in mean litter C:N ratios. *M. angustifolia* had the highest mean litter C:N ratio in spring (mean = 68.75) with comparable values found in summer (mean = 67, $p= 0.90$). Overall co-occuring native species were signifcanlty higher than *A. mearnsii* through seasons ($p<0.001$, Figure 3.5a).

At the Du Toit's River a similar trend in mean litter C:N ratios remains constant through different season in *A. mearnsii* with no signifant differences found ($p<0.01$, Figure 3.5b). In *B. stellatifolium* the highest mean litter C:N ratio concentrations in *B. stellatifolium* and was evident in winter (mean = 62.49) with differences found between winter and autumn ($p<0.01$, Figure 3.5b). Summer (mean = 59.87) and spring (mean = 62.51) are relatively similar in mean litter

C:N ratio's between the two seasons in *M. angustifolia* with autumn being siginifcanlty different from spring, summer ($p<0.001$) and winter ($p<0.05$, Figure 3.5b).

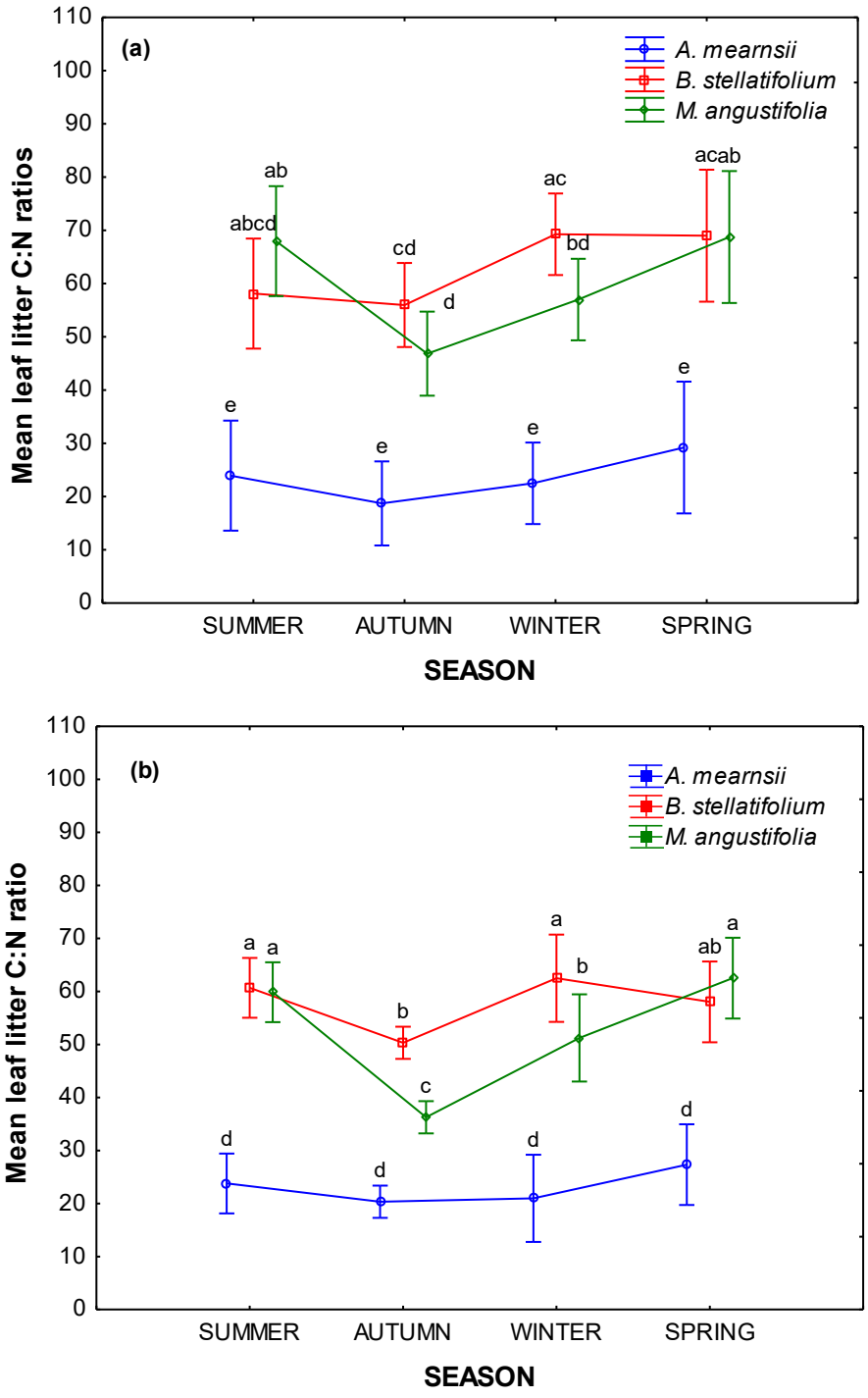


Figure 3.5: Mean litter C:N ratios concentrations (%) for *A. mearnsii* and co–occuring native species growing in near pristine and invaded areas in riparian zones at the (**a**) Wit River and (**b**) Du Toit's River. Letters denote significant differences (LSD test, $p<0.05$) based on a two way repeated measures ANOVA's (**a**) ($F_{[6.18]} = 1.44$, $p < 0.05$; (**b**) $F_{[6.18]} = 4.25$, $p < 0.01$) using all the data collected over different seasons.

At the Wit River, *A mearnsii* produced six times more mean annual leaf litter N concentrations than both *B. stellatifolium* and *M. angustifolia* (Table 3.2, site a). Additionally mean annual litter C:N ratios were three times more in the invasive *A. mearnsii* than both co–occuring native species. Similarly at the Du Toit's River, *A. mearnsii* produced six to seven times more mean annual leaf litter N concentrations in the invaded site than the co-occuring native species at the near pristine plots.

Table 3.2: Mean annual leaf litter N concentrations (mg $g^{-1}y^{-1}$) and mean annual C:N ratio in near pristine site for *B. stellatifolium* and *M. agustifolia* (N = 5) and in the invaded site for *A. mearnsii* (N=10) at the (**a**) Wit and (**b**) Du Toit's River. Values are represented as mean [± SD].

-	N	Mean annual litter N concentrations (mg $g^{-1}y^{-1}$)	Mean annual litter C:N ratios
Site (a): Wit River		mean ± SD	mean ± SD
Near pristine site:			
B. stellatifolium	5	4.03 ± 1.11	63.09 ± 3.51
M. angustifolia	5	4.84 ± 0.52	60.14 ± 5.18
Invaded site:			
A. mearnsii	10	25.28 ± 2.27	23.59 ± 2.17
Site (b): Du Toit's River			
Near pristine site:			
B. stellatifolium	5	3.10 ± 0.28	57.88 ± 2.68
M. angustifolia	5	3.61 ± 0.42	52.47 ± 5.97
Invaded site:			
A. mearnsii	10	24.35 ± 1.37	23.11 ± 1.60

3.5.2. Foliarδ^{15}N signatures in green leaves of *A. mearnsii* and native plant species in the invaded riparian zones

At the Wit River invaded site, *A. mearnsii* had the highest δ^{15}N (1.69%) between *B. stellatifolium* (1.26%) and *M. angustifolia* (1.46%) respectively (Figure 3.6a). The lowest percentage δ^{15}N were found in both natives' species with a minimum of 0.17% in *B. stellatifolium* and 0.15% in *M. angustifolia*. However, *A. mearnsii* lowest values was almost 5 times more than both native species (Figure 3.6a). In mean δ^{15}N percentages, *A. mearnsii* was significantly (1.15 ± 0.33%, $p<0.05$) different from *M. angustifolia* (0.69 ± 0.39%) but not significantly different ($p=0.05$) than *B. stellatifolium*. The Du Toit's River, a similar trend in δ^{15}N percentage are observed. *Brabejum stellatifolium* had the highest perentage δ^{15}N in it's leafs of (0.68%) and *M. angustifolia* (0.65%) for the native species, with *A. mearnsii* having the highest values between species (1.20%). The

lowest $\delta^{15}N$ percentage in leafs was recorded in *M. angustifolia* (0.10%), followed by *B. stellatifolium* (0.20%) and then *A. mearnsii* with a minimal value of 0.78 %. *A. mearnsii* had the highest mean $\delta^{15}N$ percentages (1.20 ± 0.30%) and was found to be significantly different from *B. stellatifolium* (0.68 ± 0.26%, $p<0.01$) and *M. angustifolia* (0.65 ± 0.35%, $p<0.001$) respectively.

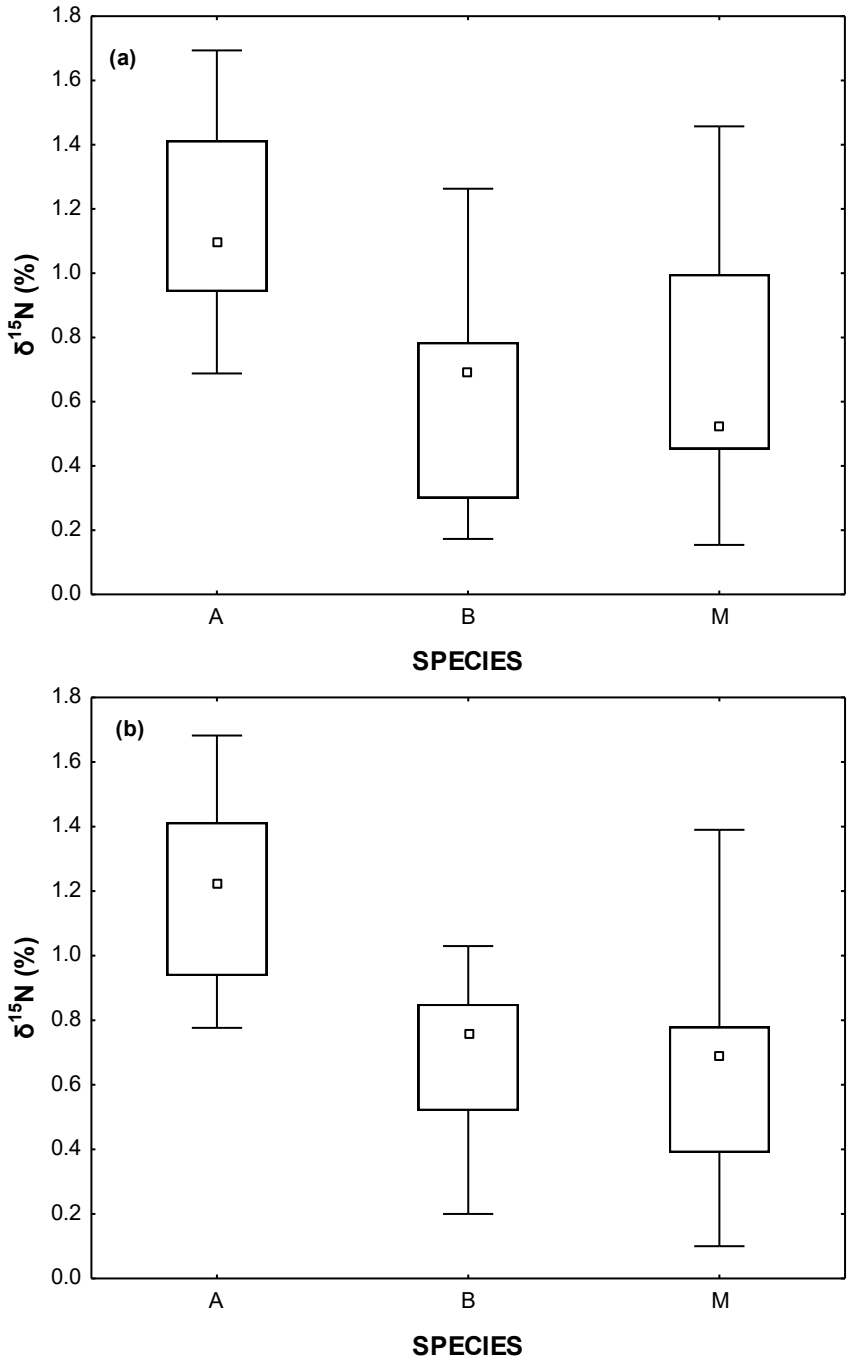


Figure 3.6: Difference in foliar $\delta^{15}N$ (%) between (A) = *A. mearnsii*, (B) = *B. stellatifolium* and (M) = *M. angustifolia* (N = 10) of their fully expanded mature leaves collected during April 2016 from the (**a**) Wit and (**b**) Du Toit's River within the invaded riparian zone. Values represent medians and whiskers indicate the minimum and maximum values.

3.5.3. Leaf nutrient content and resorption efficiency (N and P) in aboveground components of *A. mearnsii* and co-occuring native species

The mean nutrient concentrations in the mature green leaves sampled during autumn (early April), varied significantly between species at both Wit River and Du Toit's River sites. Green leaf N concentrations varied from a mean of 27. 08 mg g^{-1} for *A. mearnsii*, and a mean of 8.02 mg g^{-1} for *B. stellatifolium* to a mean of 8.76 mg g^{-1} for *M. angustifolia* at the Wit River (Table 3.3, site a). P concentrations in mature leaves of *A. mearnsii* was almost seven times higher than both native's species of *B. stellatifolium* (0.44 mg g^{-1}) and *M. angustifolia* (0.46 mg g^{-1}). A similar trend was observed at the Du Toit's River sites where mature green leaves varied significantly between species. Green N concentrations varied from 27.48 mg g^{-1} for *A. mearnsii*, 8.34 mg $g^{-1}$for *B. stellatifolium* and 8.72 mg g^{-1} for *M. angustifolia* and variation in P concentrations was also apparent. P concentrations varied from as high as 1.00 mg g^{-1} to 0.52 mg g^{-1} and 0.58 mg g^{-1} between species (Table 3.3, site b).

Green mature leaf N concentrations differed from senesced leaf N concentrations at both sites (Table 3.3). A similar pattern was observed in P concentrations, where senesced P concenrtations was much lower in P for both native species than the green leaf P concentrations. For example, in the Wit River, B. *stellatifolium* P in senesced leaf was three times (0.01 mg.g^{-1}) lower than green leaf P (0.44 mg g^{-1}). However, the variation between green and senesced leaf P in *A. mearnsii* was not as much. At the Wit River, the difference was around 0.20 mg g^{-1}. N:P ratios in mature green leaves ranged from 18.57 to 27.05 mg g^{-1} in mean values of the three species at the Wit River and from 15.11 to 28.34 mg g^{-1} between species at the Du Toit's River (Table 3.3, site a,b). In senesced leaves at Wit River mean values ranged from 28.25 to 36.80 mg g^{-1} between species and at Du Toit's River from 36.30 to 38.89 mg g^{-1} between species. The results showed differences in N:P ratios between senesced leaves when compared to mature green leaf N:P ratios, with notably *A. mearnsii* in the Wit River showing little variation between senesced and mature green leaf N:P ratios. (Table 3.3). Senesced leaves of both *B. stellatifolium* and *M. angustifolia* had higher N:P ratios than *A. mearnsii* at both sites, while N:P ratios in *A. mearnsii* senesced and green leaves did not differ in N:P ratios.

In both study sites, *A. mearnsii* and co-occuring native species were more effective (efficient) in recycling at P resorption than N resorption (Table 3.3a, b, resorption efficiencies). Calculated on a mass basis, at the Wit River nitrogen resorption efficiency (NRE) from senesced leaves for *A. mearnsii* were 25%, *B. stellatifolium* 56% and *M. angustifolia* 51%. On average, *A. mearnsii* translocated a quarter of N contained in the leaves for re-use after senescence and *B. stellatifolium* and *M. angustifolia* translocated half of their N in leaves after senescence at the Wit River (Table 3.3,site a). A similar reuse of N after senesced was observed at the Du Toit's River for *A. mearnsii* (37%), *B. stellatifolium* (56%) and (53%) of N was retranslocated in *M.*

angustifolia (Table 5.3, siteb, resorption efficiencies). P resorption was much higher than N resorption. P resorption values ranged (from a mean of 42.64 % in *A. mearnsii*, 76.67 % in *B. stellatifolium* and 69.00% for *M. angustifolia* at the Wit River. A similar range of values were observed for the Du Toit's River, with a mean of 53.43% for *A. mearnsii*, 78.11% for *B. stellatifolium* and 79.33% for *M. angustifolia* (Table 3.3, site b). *A. mearnsii* is much more efficient in resorbing P than N at both sites. N resorption proficiency in *A. mearnsii* was four to seven times greater than the two-occuring native species at both the Wit River and Du Toit's River (Table 3.3, resorption proficiency). However, P resorption proficiency was eight times higher at the Wit River and four times at the Du Toit's River in *A. mearnsii* when compared to native species.

Table 3.3: N and P concentrations (mg g^{-1}), N:P ratios, N and P resorption efficiencies (%)/proficiencies(mg g^{-1}) green and senesced leaves for the (**a**) Wit and (**b**) Du Toit's River sites for *A. mearnsii, B. stellatifolium and M. angustifolia* (N = 5). Sample collection took place in December 2016 and values are represent as mean [± SD].

	A. mearnsii	***B. stellatifolium***	***M. angustifolia***
Site (a): Wit River	mean ± SD	mean ± SD	mean ± SD
Mature green leaf concentrations (mg g^{-1})			
Nitrogen	27.08 ± 0.41	8.02 ± 0.24	8.76 ± 0.79
Phosphorus	1.12 ± 0.19	0.44 ± 0.04	0.46 ± 0.02
N:P ratio	27.05 ± 4.31	18.57 ± 0.97	19.02 ± 1.20
Senesced leaf concentration, mg g^{-1} (Resorption proficiency)			
Nitrogen (NRP)	22.04 ± 1.46	3.54 ± 0.16	5.98 ± 0.94
Phosphorus (PRP)	0.80 ± 0.20	0.01 ± 0.00	0.14 ± 0.02
N:P ratio	28.25 ± 1.57	35.40 ± 1.57	36.80 ± 2.03
Resorption efficiencies (%)			
Nitrogen (NRE)	18.60 ± 5.27	55.79 ± 1.90	50.56 ± 3.40
Phosphorous (PRE)	42.64 ± 2.67	76.67 ± 1.67	69.00 ± 6.00
Site (b): Du Toit's River			
Mature green leaf concentrations (mg g^{-1})			
Nitrogen	27.48 ± 1.10	8.34 ± 0.37	8.72 ± 0.34
Phosphorus	1.00 ± 0.11	0.52 ± 0.10	0.58 ± 0.02
N:P ratio	28.34 ± 1.94	17.71 ± 2.20	15.11 ± 0.80
Senesced leaf concentrtions, mg g^{-1} (Resorption proficiency)			
Nitrogen (NRP)	17.24 ± 1.41	3.60 ± 0.18	4.06 ± 0.19
Phosphorus (PRP)	0.46 ± 0.07	0.10 ± 0.00	0.12 ± 0.02
N:P ratio	38.89 ± 2.56	36.00 ± 1.84	36.30 ± 4.12
Resorption efficiencies (%)			
Nitrogen (NRE)	25.11 ± 4.02	56.15 ± 4.19	53.47 ± 1.19
Phosphorous (PRE)	53.43 ± 4.94	78.11 ± 3.63	79.33 ± 3.23

3.6. Discussion

3.6.1. Monthly, seasonal leaf litterfall rates of *A. mearnsii* and co-occuring native species in near pristine and invaded riparian zones

The results agree with previous findings (Milton, 1981; Witkowski 1991a; Yelenik et al., 2004; 2007; Inagaki and Ishizuka, 2011; Naude, 2012) that found greater litterfall in invasive *Acacia* species when compared to native vegetation. It is evident that the growth strategy of *A. mearnsii* is distinctively different from the co–occurring native species as there are two leaf litterfall peaks in *A. mearnsii* and one in both co-occuring native species. The results presented for leaf litterfall at the Wit River (273.66 ± 37.76 g m^{-2}) and Du Toit's River (321.32 ± 58.93 g m^{-2}), are in range with other *Acacia* species, for instance, *A. cyclops* (377.52), *A. longifolia* (335.03), *A. melanoxylon* (318.80) and *A. saligna* (488.83) in the study of Milton (1981) in the Southern Western Cape. In a later study by Witkowski, (1991a), on the SW Cape (coastal lowlands) it was found that bimonthly leaf litterfall of *Acacia* species (*A. saligna* and *A. cyclops*) was greater than the native vegetation. However, the Witkowski, (1991a) study is not comparable with Milton (1981) and the present study as it was done on a bimonthly basis, however, when converted to monthly figures, it approximates the finding from the current study (178.35 g m^{-2} for *A. saligna* and 201.72 g m^{-2} for A. *cyclops*). In later study north of Cape Town in the Western Cape Yelenik et al., (2007) found that *A. saligna* had a fourfold higher litterfall rate (404.16 ± 35.1 g m^{-2} y^{-1}) than fynbos vegetation (102.26 ± 16.8 g m^{-2} y^{-1}). The results of Yelenik et al., (2007) however was based on litterfall dropped and not leaf material only as in the present, thus suggesting that the N contribution from *Acacia* spp., are significant in leaf litterfall as first thought and showed empirically in the present study. In addition, Saharo and Watanbe, (2000) in Sumatra, Indonesia found that leaf litterfall for *Acacia dealbata, which* is a closely related species to *A. mearnsii* to be in a similar range (446 g m^{-2}) as the present study. In a recent study by Naude, (2012) in riparian zones within the south-western Cape region found in invaded sites (*A. mearnsii* and *A. longifolia*) litter mass on the ground was twice as much as the fynbos vegetation. Furthermore, the co-occurring native species in near pristine sites were found to produce considerably less litter (Milton 1981; Witkowski, 1991a; Yelenik et al., 2007), which suggests that displacement of native spcies with invasive *A. mearnsii* will lead to significant alterations to ecosystem ecology of Fynbos riparian zones.

Additionally, the results found for *A. mearnsii* and co-occuring natives were not based on all production parts (i.e., flowers, seeds, twigs). It is important to note that due to the structural nature of native and invasive plant communities (clusters versus dense stands), the data presented could be biased as native species trap was placed under single trees and *A. mearnsii* under dense stands and might be an overestimation of leaf litterfall for the respective species.

3.6.1.1. Leaf litterfall and nutrient (N and C) return inputs from *A. mearnsii* and co-occuring native species in riparian zones

The temporal trends in leaf litterfall rate of *A. mearnsii* demonstrate two peaks one in the dry season April (mid-autumn) and one in December (mid-summer) for both *A. mearnsii* and co-occuring native species. Leaf litterfall peak in autumn (April) is postulated to be the senesced stage of *A. mearnsii* just before the winter season. Therefore, *A. mearnsii* effectively recycles nutrients in autumn, which coincide with new leaf growth as the wet season approaches (Chapin and Shaver, 1989; Tye, 2013). The leaf litterfall rate in December in *A. mearnsii* coincided with the flowering season of the species (see appendix A). Many authors (Moncur et al., 1988; Campbell, 2000; Nyoka, 2003; Dell'Porto et al., 2006) stated that the flowering season of *A. mearnsii* are in the months of September-November, which occurs generally for 8 to 10 weeks, however, in the present study leaf litter coincided with flowers in late December and into January. Moncur et al., (1988) and Bonney, (2003) described this as an irregular period for *A. mearnsii* growing in Australia. Similarly, the co–occurring species of *B. stellatifolium* and *M. angustifolia* has one leaf litter stage in December, which is the stage of senescence of the species.

It is sensible to presume that *A. mearnsii* is less reliant on seasonal retranslocation of N than co–occurring native species (Tye, 2013). Richardson et al., (2009a) indicated that warm temperatures increases N mineralization rates, which can lead to increase plant uptake and elevated levels of foliar N and was found in Tolsma et al., (1987) and Fife et al., (2008). The high mean leaf litter N concentrations of *A. mearnsii* are due to the ability of the species to fix N and this found in other studies (Tye and Drake, 2012; Van der Colff et al., 2017). To overcome nutrient limitation (notably N) *A. mearnsii* mine nutrients through strategies such as specialized root systems and extensive mycorrhizal networks which allows the species to keep high N levels in their leaves throughout the year (Lambers et al., 2006; Potgieter, 2012; Tye, 2013). The results found agreement with previous findings by Tye (2013) who found similar ranges of mean annual N concentrations (in leaves) between seasons for *A. dealbata* in the Mpumalanga Province, South Africa. Additionally, the early leaf emergence and longer maintance of green leaves in *A. mearnsii* compared to native species gives them an advantage in transporting nutrients to the roots for storage to ensure better winter survival and spring growth (Richardson et al., 2009a; Tye, 2013). Native species tend to produce leaf material with lower year round nutrient concentrations and longer lifespans. Results found in other studies (Yelenik, 2004; Yelenik et al., 2007; Juba 2012 unpublished; Tye and Drake, 2012; Maoela; 2015;) for co–occurring native species agree with the low N concentration found in the present study. A good account of the nutrient conservation strategy of the co–occurring native species is the high litter N concentrations of both species in summer (December). This is the season where allocation of

nutrient's are at its maximum as the plant is resorbing nutrients for reuse before leave senesced (Aerts, 1996; Chapin and Shaver, 1989; Tye, 2013). Besides the nutrient inputs (N) which is expected from N_2-fixing plants (Tye, 2013) there are other ways that *A. mearnsii* or invaders can alter nutrient cycling (Naude, 2012; Tye, 2013; Van der Colff et al., 2017). A good example of this is mineralization rates, which are reliant on C:N ratios of leaf litter. If there are difference between species (invasive vs native) difference nutrient cycling rates are expected which is reliant on C:N ratio's (Tye, 2013; Van der Colff et al., 2017). Leaf litter of low C:N ratios likely decompose faster than leaf litter of higher C:N ratios and as a results it will cause a shift in mineralization rates between species (Killingbeck, 1996; Tye, 2013). This is evident in mean leaf litter C:N ratio of the co-ocuring native species which has greater C:N ratios in their leaves compared to *A. mearnsii*. It was difficult to compare the seasonal nutrient data (C and N) to other studies simply because values are scarce in literature for both invasive and native aboveground components, He et al., (2011) in Australia, Tye, (2013) in South Africa also found it difficult to compare there data to other studies.

The high foliar N concentration in leaf litterfall in the riparian zone by *A. mearnsii* enhances nutrient concentrations especially N in soils under *A. mearnsii* stands. The increase in soil N is expected as *A. mearnsii* was not limited by N supply as the species is a nitrogen fixer (Tye and Drake, 2012). Even though no soil N data is presented in this study for *A. mearnsii*, there is a considerable amount of literature to support the argument that N_2-fixers notably *Acacia* spp. effects terrestrial and and riparian soil N stocks in the south-western Cape (Witkowski, 1991a; Yelenik et al., 2004; 2007; Naude, 2012). In addition, the same findings were found by Marchante et al., (2008) in the dune ecosystems of Portugal and later on by Hellmann et al., (2011) which reported increased available inorganic N in uninvaded environments for *A. longifolia*. At ecosystem level, the effect of N_2-fixing plant species on nitrogen pools has been documented in many parts around South Africa and the world (Stock et al., 1995; Marchante et al., 2010; Naude, 2012; Tye and Drake, 2012). Thus, the significant amount of N rich leaf litter that *A. mearnsii* produce can affect riparian zone soils (riparian biochemistry) and eventually add N to aquatic environments as allochthonous inputs and when proceeded could alter aquatic environments.

3.6.2.1. *A. mearnsii* leaf $\delta^{15}N$ signatures at invaded riparian zones

All the *A. mearnsii* samples at the Wit and Du Toit's River invaded riparian zones had positive $\delta^{15}N$ (‰) signature, and significantly higher than the native species, which was an indication that *A. mearnsii* access a substantial amount of soil N (Jacobs et al., 2006). The key determination of leaf N isotope composition is the isotope ratio of the external N source and plant mechanisms (Evans, 2001; Tye, 2013). One explanation for this trend is the root-architecture of the native and invasive species. Many invasive *Acacia* spp. in southern Africa are deep-rooted species,

which is a physiological function to overcome water and nutrient limitation in arid and semi-arid environments (Handley and Raven, 1992; Morris et al., 2011; Tye, 2013). The deep-rooted *Acacia* spp. can access a diverse N pools even take up the same form of N as species surrounding them (i.e, NH^4). Shearer and Kohl, (1986) and Högberg, (1997) indicated that with increasing depth in the soil profile, $\delta^{15}N$ increases. The pattern is the combined effects of ^{15}N enriched of organic matter due to microbial decomposition in deep soils, the remoteness of N novel inputs and lastly the production of fresh litterfall that is depleted in ^{15}N in relation to the soil N (Högberg, 1997; Tye, 2013). The coherent effect of these processes suggests that invasive *Acacia* spp. through their deep roots may be taking up N with a $\delta^{15}N$ that is enriched in relation to a shallow rooted species. For example, in a field experiment in Hluhluwe-iMfolozi Park at Kruger National Parrk, Cramer et al., (2007) reported that deep-rooted *Acacia* spp. had more positive $\delta^{15}N$ values than shallow rooted species.

An alternative explanation may be that the cycling of N under invasive plant canopies may be higher than within native communities, which also suggests that the N cycle may be more open, i.e. more prone to fluxes out of the system. Isotopic fractionation can take place at different stages (uptake, transport or assimilation) of a plants N cycling and is different between plants using different strategies at each step (Craine et al., 2015). Sites with high N availability are more likely to have with plants with higher N concentrations, which is associated with positive plant $\delta^{15}N$ signatures (Martinelli et al., 1999; Cramer et al., 2007). Additionally the resorption of nutrients particularly N from leaves can lead to $\delta^{15}N$ positive enrichment signatures of leaf N due to preferred resorption of the light isotope, which happens through the metabolic fractionation (Tye, 2013). A third explanation may be found in the symbioses that the various plants engage in. A good account of this is plants associated with different types of mycorrhizal fungi facilitate N acquisition from the soil will have different $\delta^{15}N$ values (see review by Boddey et al., 2000). *Acacia mearnsii* acquired inorganic N from the soil in the present study. In a study by Goi et al., (1993) *A. auriculiformis* acquired a high uptake from soil N that resulted in increased leaf $\delta^{15}N$ signatures. However for the *Acacia* spp (*A. saligna* and *A. cyclops*) in the CFR region, Stock et al., (1995) found relatively low or negative values in these species, suggesting that the invasive plants could have used different stages in the uptake, transport or assimilation of N. The positive values found in Goi et al., (1993) and Martinelli et al., (1999) were related to greater isotopic fractionation in a more N–replete system. The addition of N rich litterfall in autumn and the decomposition of the litter which is N riched could have contribute to an N repleted system (N rich) which repleted the invaded environment and eventually taken up by the plant (Evans, 2001; Tye, 2013).

3.6.3. Resorption efficiencies in competing *A. mearnsii* and co–occurring native species

The co–occurring native species effectively recycles N and P nutrients before leaf abscission at both the Wit and Du Toit's River, which was higher than the global average reported for (P 52%) and (N 50%) by Aerts, (1996). P resorption efficiencies in co-occuring native species were higher than 34 evergreen species (mean = 34%) reported by Tang et al., (2013) in China. However, Van der Colff et al., (2017) found similar resorption efficiencies of N nd P in the Garden Route National Park in the native species *Virgilia divaricata.* The two co-occuring native species of *B. stellatifolium* and *M. angustifolia* are considered key stone species and only kind of their genus (Galatowitsch and Richardson, 2005; Crous, 2010) on the old, climatically buffered, infertile landscapes (OCBLS) as defined by Lambers et al., (2010). Therefore plants on these soils (ancient inhabit oligotrophic soils, Power, 2010) develop long-term adaption strategies and employ root adaptions such as root clusters to assist to assist in P-acquisition, which is effectively a 'mining' strategy for nutrients (Lambers et al., 2010). Clusters roots can readily access soluble P from the highly weathered and leached ancient soils from regions like Australia and the Fynbos biome in South Africa as species with mycorrhizal symbioses (N_2-fixing plants) (Lambers et al., 2008b; Power, 2010; Potgieter, 2012). Therefore, P acquisition is a species-specific adaption strategy to mine and remobilize nutrients effectively, which makes them good nutrient conservation species (Aerts, 1995; Aerts and Chapin, 2000; Wright et al., 2004). However, plants tend to remobilize nutrients (N and P) more efficiently when it is difficult to acquire from soils that are nutrient poor and this was found in other studies (Wright et al., 2004; Het al., 2011; Tye, 2013). The native species both posess sclerophyllous leaves, a characteristic of fynbos plant species (Maamri et al., 1994, 2001) which have longer life spans than non-sclerophyllous leaves (invader species). Therefore they can extend the nutrient preservation time to conserve nutrients as observed in the leaf sensesced stage in summer (Wright and Westoby, 2003; Tye, 2013). The high P and N resorption efficiency values in the native species exhibit the true maximum resorption potential of the species because of the long-term adaptation to impoverished soils as the Fynbos biome.

A. mearnsii cycles a significant amount of P before leaf abscission. However, it is not as efficient in cycling N. Symbiotic nitrogen fixation has a high demand for P as 20% of plant P is distributed to nodules as N_2-fixation takes large quantity of energy to access a larger pool of P (Allsopp and Stock, 1992; Schulze et al., 1999, Potgieter, 2012). P may also be acquired by Australian *Acacia* spp*.,* through their extensive root systems and greater mycorrhizal symbionts that allow them to enlarge the soil volume exploited and increase the number of places for mycorrhizal establishment to enhance acquisition of P and other nutrients (Hoffman and Mitchell, 1986; Power, 2010). In addition, Naude, (2012) found that acid phosphatase activity under *A.*

mearnsii stands in the Wit River valley was significantly higher than the native areas, which suggests another avenue for acquiring P. In the south-western Cape in the CFR, Witkowski, (1994) found that *A. saligna* root penetration was faster than *A. cyclops* over a month period, which potentially could have assist them in tapping into the water table. Cramer et al., (2009) stated that greater water availability, could contribute to P–acquisition via mass flow, which may apply to e.g. riparian environments. However, the higher P resorption than N suggests that despite these multiple strategies, P may still be a limiting nutrient for N-fixing *Acacia* species. Nonetheless, these characteristics to satisfy their P demands likely contribute to their success in the Fynbos biome and particularly in *A. mearnsii*. Most recently Van der Colff et al., (2017) in a study located within the Garden Route National Park, South Africa also reported higher P resorption than N resorption in *A. mearnsii*. P resorption efficiencies reported in the study for *A. mearnsii* are lower than values reported in a study by He et al., (2011), who found up to 80%, for arid acacia species in the North Western Australia. The resorption of P reported in the study is in the same range as global average (56%) for woody plants, which was reported by (Aerts, 1996; van Heerwaarden, 2003).

Many authors (Evans et al., 1989; Lima et al., 2006; Siddique et al., 2008) indicated that high N soil availability leads to low nitrogen resorption efficiencies. This is postulated to have occurred as *A. mearnsii* acquired inorganic mineral N from the soil as a significant amount N rich leaf litter return to soil under the dense stands of *A. mearnsii* at the invaded riparian zone in December. The species may possibly be increasing N supply from the roots (N availability) and therefore shift between a crossover point from symbiotic N_2-fixation to N resorption efficiency which leads to decline in N nutrient efficiency as stated by Tye, (2013). However, the abilility to fix nitrogen does not mean an inability to resorb nutrients (N or P), which suggest that there is no direct relationship (Killingbeck 1993a; Houlton et al., 2008; Inagaki et al., 2011; Tye, 2013). Van der Colff et al., (2017) stated even under a changing nutrient environment, *A. mearnsii* retains its nutrient resorption efficiency. The low N resorption efficiencies for *A. mearnsii* in the present study are found in other studies for IAP's (Cote and Dawson, 1986, Cote et al., 1989, Killingbeck, 1993a; Tang et al., 2013). Plants capable of N_2-fixation tend to have lower N-resorption resorption efficiencies than non-N fixing species, with no difference in P resorption efficiency (Killingbeck, 1996; He et al., 2011).

There is a debate whether Mediterranean ecosystems such as the CFR in the Fynbos biome is limited by N or P (Sardans et al., 2004; Diaz et al., 2012; Potgieter, 2012). To answer this mystery, the model of Killingbeck's, (1996) had to be taken under consideration as the model defines resorption proficiency in terms of senesced leaf as the ultimate potential of plant nutrient resorption. The N resorption proficiency in *A. mearnsii* is consistent with other fined leaved legumes growing in the Komati Wildlife Reserve in Mpumalanga (mean 18. 43 mg N g^{-1},

Tye, 2013) and savannas landscapes (mean of 18 mg N g^{-1}; Ratnam et al., 2008). Additionally the values found for *A. mearnsii* are also greater than *Acacia* spp. growing in the Australian Great Sandy Desert (13 mg N g^{-1}; He et al., 2011) and on par with global average (16.1 mg N g^{-1}) for N-fixing species as noted by Killingbeck, (1996). The P resorption proficiency in *A. mearnsii* are much higher than the threshold for complete resorption in evergreen species (< 0.4 mg g^{-1}) and inparticular to its close relative *A. dealbata* (0.13 mg N g^{-1}, Tye, 2013). It suggests that higher P resorption proficiencies in *A. mearnsii* may help them inhabit impoverished sites or landscapes. The difference between species in proficiencies of P are the likely cause to bring ecosystem-level changes due to ehance nutrient availability (N and P) in riparian and adjacent stream and when proceeded and can be detrimental for aquatic environments in Fynbos Rivers. Overall, it indicates that the species in the riparian zones at Wit and Du Toit's River conserve P more tightly than N, indicating a P limitation, which has been found in other studies (Ratnam et al., 2008; He et al., 2011; Tye, 2013; Van der Colff et al., 2017). This limitation is a key characteristic of southeastern Australia (He et al., 2011) and South African landscapes (Lambers et al., 2008b; Tye, 2013; Van der Colff et al., 2017) as these species both undergone species specific adaption strageties to the landscape to acquire nutrients. Determining the mineral nutrition between invasive and native species in an ecosystem, may help us understand the great success invasive plants particularly N_2-fixing plants has over native plant species in the Fynbos biome of South Africa where they are most widespread (Le Maitre et al., 2002).

It is highly important to acknowledge when comparing the present results (nutrient resorption efficiencies) to other studies that the current estimates for resorption efficiency are articulated on a mass basis and not leaf mass area (LMA) as done by other authors (Killingbeck, 1996; He et al., 2011). The calculation of leaves on a mass basis instead of leaf area basis has its advantages. A good example of this is calculating the leaf on a mass basis mean a reduced influence of the loss of the leaf mass area during senesced stage whereas expressing it on an area basis can accurately represent net nutrient movement from senescing leaves. *A. mearnsii* has a relative small leaf mass area and subject to considerable error as it could be complicated to measure. Therefore, in the study it was chosen to limit the expression of resorption efficiencies to mass basis (concentrations on mass basis) and distinguish that our mass based estimates are likely to overestimate resorption efficiencies (He et al., 2011; Tye, 2013). However, due to the reduction in leaf mass during senescence is generally less than 10%, which makes the errors marginally small.

3.7. Conclusion

It is well documented that IAP's (Ehrenfeld, 2003; Yelenik et al., 2004, 2007) alters nutrient cycling of localised ecosystems, especially if the invader species is different from the native species in its nutrient use strategies. To our knowledge this work demonstrate that the invasion

of *A. mearnsii* into the South African Fynbos biome is supplement and accompanied by an alteration in the nutrient cycling, most specifically notably nitrogen. Inclusive taken together, there is a surfeit of evidence that the invasion of *A. mearnsii* has the potential through nutrient inputs (litterfall) to alter the N cycling of a landscape and P cycling to a less significant but noticeable significant extend. The ability to constantly shed nutrient rich leaf (N and possibly P) are seen as another pathway for the species to cope with limitations in the landscape to which studies have not yet detected in the highly plant diverse area of the Fynbos biome. Additionally this 'hunger' for nutrients by *A. mearnsii* potentially is the main reason for its success in the Fynbos biome. Further, allochthonous inputs of N and P litter can be transferred and further downstream and alter aquatic environments. These bioactive alterations could be detrimental for ecosystem away from the point of invasion and add to the already expensive cost to river restoration programmes like the WfW to remove the woody invasive aliens especially in the Fynbos biome where they are most prevalent.

Chapter 4

Decomposition rates of *A. mearnsii* compared to native woody species and macroinvertebrate communities of native and invasive litter in the mountain streams of the Fynbos biome

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4.1 Abstract

Inputs from leaf litter are essential source of nutrients for aquatic food webs, which are dependent on the organic matter provided by riparian vegetation. However, the invasion by alien trees in riparian zones could lead to alterations in the amount and timing of leaf litter inputs to freshwater ecosystems, as well as altered nutrient dynamics through modified decomposition rates. The home-field advantage hypothesis (HFA) posits that leaf litter from a plant in the place of origin (home) will decompose much quicker by macroinvertebrates than litter that has been translocated. In this study, the HFA hypothesis was tested in two perennial rivers in the Fynbos biome of South Africa, a global biodiversity hotspot. Leaf litterbags was placed in streams with similar characteristics and riparian vegetation cover, in two river reach types: near-pristine fynbos (natural vegetation) and reaches invaded with black wattle (*Acacia mearnsii*), an invasive N-fixing tree species from Australia. Bags were collected after 14, 28, 48, 64, 80 and 102 days of incubation of both fynbos and *A. mearnsii* species litter. The amount of remaining litter (AFDM %), and decomposition rates and macroinvertebrates assemblages on leaf bags between native species and *A. mearnsii* were determined in near pristine and invaded reaches, to test HFA effects and macroinvertebrate litter infinity effects. The results found invasive species to decompose at a much faster rate than fynbos species, which was explained by differences in litter quality (N and C:N ratio) between species, rather than the HFA hypothesis. The two rivers and each reach were homogeneous in stream characateristics and thereby litter decomposition was related to plant nutrient content. Macroinvertebrates assemblages was regulated by site factors and selective pressure for resources on certain seasons and not HFA macroinvertebrate litter affinity effects.

KEYWORDS: litter inputs; riparian vegetation; nutrient dynamics; home-field advantage; aquatic ecosystems; litter quality; instream communities; function and structure

4.2 Introduction

In aquatic environments, leaves decompose at different rates, which is dependant on both internal and external factors (Petersen and Cummins, 1974; Webster and Benfield, 1986). Internal factors are mainly the difference in leaf litter nutrient content (C, N and P) and structural properties such as leaf shape, as well as the composition and abundance of the macroinvertebrate communities (Webster and Benfield, 1986; Reinhart and VandeVoort, 2006). The external factors are stream characteristics (temperature, flow regime, physical abrasion, and substrate) which is suggested to be different in upstream and downstream reaches and different microhabitats (pools, runs and riffles) within a river ecosystem and therefore different decay rates are found (Maamri et al., 2001;Sponseller and Benfield, 2001; LeRoy et al., 2006;). There are a few studies in the Fynbos biome (King et al., 1986; 1987) that assessed the environmental factors that may influence litter breakdown. However, to unravel the effects are not easy as there is variation in effects such as site characteristics, litter quality differences between species and variation in decomposer communities in Fynbos biome rivers (Bengtsson et al., 2011, 2012). Therefore, information on the processes and factors influencing decomposition in freshwater environments of mountain streams in the Fynbos biome remains relatively sparse.

Inputs of leaf litter from different plant species are different in structure and chemical properties such as leaf shape, different N concentrations, carbon: nitrogen ratio (C:N) and lignin concentrations (Ayres et al., 2009). Litter input is seen as a major vector moving energy and nutrients for biota in aquatic ecosystems (fungi, bacteria, and invertebrates such as fish and macroinvertebrates) (Negrete -Yankelevich et al., 2008; Ayres et al., 2009; Kuglerova et al., 2017). The decomposition of leaf litter is explained by 70 %, which consist of the physical (i.e, leaf shape and size) and chemical composition (nutrient content) and the other 30% by in stream characteristics (LeRoy et al., 2006) and HFA effects (Gholz et al., 2000; Parton et al., 2007). In the last decade studies (Ehrenfeld, 2003; Allison and Vitousek, 2004) found that, invasive alien plant species (IAP's) which has high leaf litter composition (especially N and P) tend to decompose much faster than native species. The faster decay of leaf litter from IAP's compared to native species litter has been reported when the invasive plant were N_2-fixing plants and the native species not (Witkowski, 1991a). The physiological characteristics of IAP's, e.g. high nutrient concentrations, species specific leaf area (SLA) and N_2-fixing capabilities are key functions in faster decomposition rates when compared to native species (Allison and Vitousek, 2004; Morris et al., 2011). In contrast, slower decomposition rates of IAP's were found in other studies (Witkowski, 1991a, different site; Drenovsky and Batten, 2007). These conflicting findings suggest more effort needs to go in improving our capabilities to predict the impact of IAP's on real ecosystems in field situations (Davis et al., 2011; Furey et al., 2013). In a study by Witkowski, (1991a) it was found that litter decomposition rates of the invasive nitrogen fixer

Acacia cyclops were slower than the native species (*Pterocelastrus tricuspidatus*) in coastal lowlands of Cape fynbos in the Western Cape. In the same study in a different field setting Witkowski, (1991a) found that litter decomposition rates of the N_2-fixing *A. saligna* were faster than the co-occurring native species (*Leucospermum parile*). The differences in rates were linked to differences in litter quality characteristics between species and nutrient conservation strategies, e.g. through retranslocation of leaf nutrients during senescence (Ehrenfeld, 2003; Allison and Vitousek, 2004).

Until recently it was thought that the breakdown and mining of plant resources were mostly done by generalist consumers. However, there is a growing amount of evidence that plant species has a species specific or affinity effect to certain macroinvertebrate communities in terms of decomposition (Veen et al., 2015). The decomposer communities as a result may become adapted to and form a specialized affinity to the litter they encounter over an extended period. As a result they become efficient at breaking down their own litter matrix (absorbing nutrients), e.g. from the riparian plant community above them (Ayres et al., 2009). Consequently this “at home” benefit has been referred to as the ‘home-field advantage’ (HFA) hypothesis where litter decomposes faster in its home environment than far from its origin (Gholz et al., 2000; Ayerez et al., 2009). The specialized affinity effect macroinvertebrate communities has to certain litter types is driven by interacting drivers such as the different leaf litter quality received as input from the riparian zone and the incubation conditions (site conditions) which can be measured over weeks or months (Jewel et al., 2015; Veen et al., 2015). In literature, many other studies (Freschet et al., 2012; Veen et al., 2015) tried to test the occurence HFA, but failed to unravel the effects as the conditions until present still remains uncertain. Litter diversity in a home environment is not always associated with faster decomposition rates and macroinvertebrate litter affinity effects (Austin et al., 2014; Jewel et al., 2015; Veen et al., 2015).

There is however evidence of HFA effects where invertebrate decomposer communities become adjusted to feeding on their home turf litter but are less efficient at breaking down the foreign litter regardless of plant diversity or C:N ratios (Veen et al., 2015). The difference in effects is variable, depending on both biotic and abiotic factors of the ecosystem, which influences litter decomposition rates (Veen et al., 2015). It remains relatively unknown if the introduction of alien invasive plants species can affect macroinvertebrate litter affinity effects, and how the process of decomposition proceeds in an altered environment. To have a better understanding how and when decomposition rates and macroinvertebrate communities interrelate with litter to influence HFA effects, it is crucial to look into significant drivers of the interface between the plant community, litter type and environmental conditions at present (Freschet et al., 2012). Yet, it is unclear at the present time if litter of a different type would affect macroinvertebrate communities in a HFA microsite as vegetation peaks (leaf litterfall period) and

thereby increasing invertebrate species richness as the litter is crucial source of energy for in stream macroinvertebrate species (Buddle et al., 2006). Therefore, these mechanistic links needs added examination (Van der Wal et al., 2013). Many studies testing the HFA hypothesis across the globe (Vivanco and Austin, 2008; Ayrez et al., 2009; Jacob et al., 2010; Austin et al., 2014) have found inconsistent results, for example some have shown decomposition rates accelerated in their native and not in away or foreign environments. A study by Veen et al., (2015) suggested that the current understanding of the HFA phenomenon is not adequate to generalize across diverse systems as environmental characteristics of HFA remains unexplored. The Veen et al., (2015) study also revealed that dissimilarity between litter characteristics could be a strong predictor of HFA effects of decomposition and possibly macroinvertebrate affinity effects.

Even with the facts on the impact of invasion of *A. mearnsii* on terrestrial communities, the potential effects of *A. mearnsii* invasion on leaf processing and macroinvertebrates communities in stream remain unstudied, which limits our understanding of the highly diverse hydrological and biological components of the Mediterranean streams in the Fynbos biome of the CFR. Broadly, the study investigated the impact of *Acacia mearnsii* invasion and their impact on the ecology of mountain streams in the Fynbos biome. Here we (a) compare the decomposition rates (here used as a broadly inclusive term of both bacterial/fungal and decomposition) between the invasive *A. mearnsii* and co–occurring native species (here after “fynbos species”); (b) determine if HFA plays a role between *A. mearnsii* and fynbos species in bacterial/fungal decomposition when placed far from origin and in their native reaches; and (c) determine if HFA affinity plays a role in freshwater macroinvertebrates of *A. mearnsii* and fynbos species in their native reaches and away from origin.

4.3 Site descriptions, Methods and Materials

4.3.1. Study areas in the Fynbos biome of the CFR

The focal area for this study was in mountain and foothill stream sections of the Mediterranean-type Fynbos biome in the Breede Water Management Area (WMA), Western Cape. The vegetation in the upper catchments are typically characterised by sclerophyllous fynbos and sandstone soils, which are acidic and nutrient poor (Day and King, 1995; Samways et al., 2011). The climate is of the Mediterranean-type, which is categorized by dry summers and wet winters. After the first heavy rains, mountain rivers in the CFR have surface flow in winter while in summer smaller streams can be reduced to perennial pools and sections of riffles resulting in periodic water shortages (Goldblatt and Manning, 2000; Tharme, 2010).

Two perennial rivers were chosen for the study. The small perennial river of the Breede River, the Wit River and the tributary of the Riviersonderend River, the Du Toit's River which is cut short by the Theewaterskloof Reservoir (Tharme and Brown, 2004). Along each river, two vegetation invasion statuses were identified: (1) near pristine sites (NP); (2) invaded sites (IV). Near pristine sites were predominant with typical natural fynbos plant species (cover of *B. stellatifolium* and *M. angustifolia*) and some scattered *Searsia angustifolia* in the Du Toit's River and *Erica caffra* and *Elegia capensis* at the Wit River with no alien invasive trees present at either site. Invaded sites had more than 50% cover of *A. mearnsii*. No significant areas of commercially afforested or agricultural areas were found near the chosen study sites. Site characteristics of each invasion status at each river (Wit and Du Toit's River) are summarized within Table 4.1 and 4.2. Sampling started on the 4th November 2016 in mid-spring and endured until 6 February 2017, mid-summer.

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Table 4.1: A summary of site characteristics of the Wit River and associated invasion statuses (Near pristine and invaded) in the Breede Water Management Area (WMA). Values are represented as mean [± SD] and continuous variables were recorded periodically from the start of the field experiment until the end.

Site: Wit River	**Near pristine (NP)**	**Invaded (IV)**
Catchment elevation (m a.m.s.l.)	958.40	901.40
Reach length (m)	140	210
Wetted channel width (m)	9.46	15.30
Range of average water temperature (˚C)	14.52 to 29.15	14.80 to 29.15
Mean water temperature (˚C) [SD]	22.15 [1.92]	22.07 [1.93]
Range of discharge (m^3 s^{-1})	0.76 to 0.81	0.66 to 0.77
Mean discharge (m^3 s^{-1}) [SD]	0.78 [0.01]	0.69 [0.02]
Range of pH	4.42 to 4.74	4.41 to 4.80
Mean pH [SD]	4.61 [0.05]	4.60 [0.10]
Range of EC (µS/cm^{-1})	74.80 to 75.20	74.40 to 75.00
Mean EC (µS/cm^{-1}) [SD]	75.03 [0.06]	74.64 [0.08]
Microhabitats	Pool	Pool
Substratum characteristics	Vertical gradient, physical features including wide single channels, dominated by large boulders, medium sized cobbles with few riffles and patches of sand in certain segments of the reach, alongside the wetted channel, banks comprise of medium sized cobbles and bedrock	Substratum is dominated by small cobbles and gravel through the reach with large boulders in some section of the reach, no cascades, alongside the wetted channel banks comprises of sand, woody shrubs and riparian vegetation

Table 4.2: A summary of site characteristics of the Du Toit's River and associated invasion statuses (Near pristine and invaded) in the Breede Water Management Area (WMA). Values are represented as mean [± SD] and continuous variables were recorded periodically from the start of the field experiment until the end.

Site: Du Toit's River	**Near pristine**	**Invaded**
Catchment elevation (m a.m.s.l.)	981.50	924.20
Reach length (m)	110	130
Wetted channel width (m)	3.38	8.58
Range of average water temperature (˚C)	12.59 to 27.47	12.40 to 27.47
Mean water temperature (˚C) [SD]	18.85 [2.42]	20.43 [1.94]
Range of discharge (m^3 s^{-1})	0.16 to 0.40	0.43 to 0.71
Mean discharge (m^3 s^{-1}) [SD]	0.23 [0.03]	0.51 [0.04]
Range of pH	4.76 to 5.12	4.50 to 5.10
Mean pH [SD]	4.94 [0.05]	4.70 [0.08]
Range of EC (µS/cm^{-1})	69.30 to 74.20	70.20 to 74.40
Mean EC (µS/cm^{-1}) [SD]	72.61 [1.10]	73.56 [0.56]
Microhabitats	Pool	Pool
Substratum characteristics	Vertiginous gradient, physical features displaying features of Southwestern Cape rivers, including single, narrow channels dominated by riffles that is short and shallow and sections of cascades with deep bedrock - bottomed pools	Substratum is dominated by small rounded cobbles, gravel and small boulders, river banks is dominate with woody shrubs and sand that is relatively high in certain segments and throughout the reach major biotopes are pools and occasional backwater with no cascades

4.3.2. Experimental set up for leaf litter decomposition and macroinvertebrates

Leaves of *A. mearnsii* and two native species, *B. stellatifolium* and *M. angustifolia* were collected from single trees in December 2015 and November 2016 just before abscission or shortly after they had fallen (Norbly et al., 2000). All leaf material was oven dried at 50 °C for 24 h. To test for differences in decomposition and macroinvertebrates between leaf types, and invasion status (NP or IV) leaf bags were imbedded at both rivers (Wit and Du Toit's River at the different invasion sites) from 4 November 2016 to 6 February 2017 (incubation periods of 14, 28, 48, 64, 80 and 102 days). Leaf bags were made out of nylon fine mesh (0.5 mm) for the decomposition experiment to exclude macroinvertebrates (Webster and Benfield, 1986) and macroinvertebrate bags were made out of fine – nylon coarse mesh (0.5 mm with a 2 mm screening window) to allow access to macroinvertebrates (Figure 4.1). Decomposition experiment bags was design was adjusted that decomposition of leafs were driven by in stream characterstics (bacterial/fungal) and leaf composition (Graça et al., 2001). The surface area of leaf bags for both experiments was 0.04 m^2. A total of 5.0 g of *A. mearnsii* leaves was placed into litter bags, and for the native species litter was weighed out to 2.5 g for each species, mixed and placed into litter bags, hereafter called 'Fynbos species'. A total of 384 (192 per river system) leaf bags were fabricated for the decomposition and macroinvertebrate experiments.

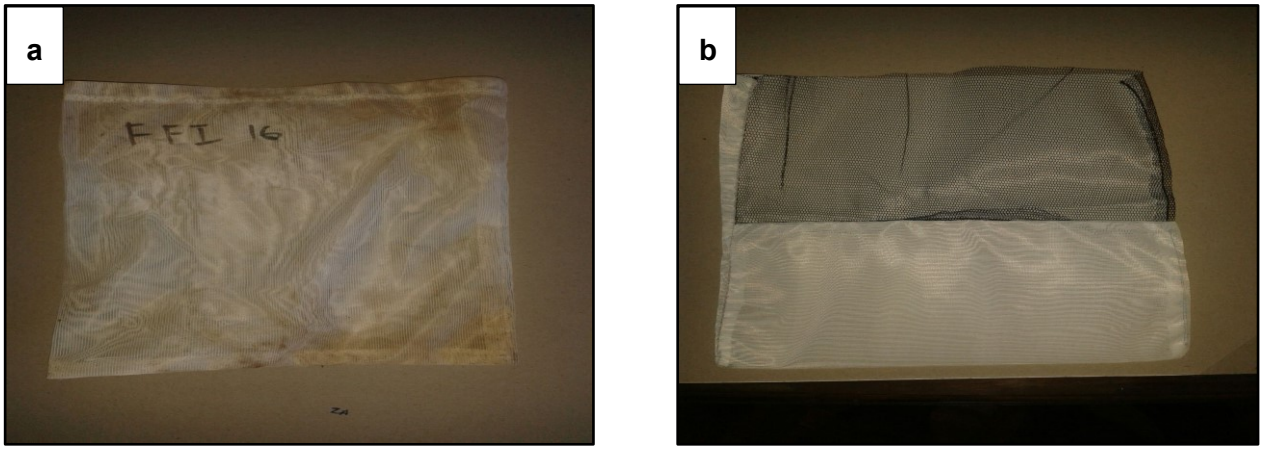

Figure 4.1: Representation of leaf bags made out of (**a**) nylon fine mesh, 0.5 mm (exclude macroinvertebrates) and leaf bags made out of half (**b**) nylon fine and coarse mesh, 0. 5 mm with a 2 mm screening window (included macroinvertebrates).

The home field advantage (HFA) hypothesis states that in a 'home' environment the decomposition of the leaf litter would generally be faster than away from it and that macroinvertebrates favours the home turf litter 'litter affinity effect' (Austin et al., 2014). Fynbos species litter (HFA) and *A. mearnsii* (HFA) decomposition bags were placed in their native reach (i.e. near pristine = fynbos species (HFA); foreign litter which was the *A. mearnsii* litter bags) and in the invaded site. The same procedure was carried out for the macroinvertebrate experiment to test the HFA hypothesis and whether macroinvertebrates will favour its home turf or introduced

litter. At every incubation date, eight bags per invasion status (NP or IV) were retrieved which consisted of the HFA leaf bags and foreign leaf bags. The same retrieval procedure was followed for the macroinvertebrate experiment. At the initial placement of bags all 384 leaf bags (nylon fine mesh, 0.5 mm which exclude macroinvertebrates and fine-coarse mesh, screening window which included macroinvertebrates) was placed at each site and different. In total 32 bags were retrieved per river system at every incubation period with unique codes (Table 4.3). Four leaf bags (of both experiments) were tied to a steel rod with gutter nails at each reach. The steel rod was used as an anchor against any obstruction that might flow downstream due to strong flow variability (Webster and Waide, 1982).

Table 4.3: Retrieval schedule for leaf bags of decomposition and macroinvertebrates experiments leaf bags, in near pristine and invaded reaches at the Wit River and Du Toit's River sites. HFA = the species in its home environment.

Study site:	**Wit River (a)**	**Du Toit's River (b)**
Retrieval schedule	Decomposition	
Near pristine reach:		
Fynbos species (HFA)	4 leaf bags	4 leave bags
A. mearnsii	4 leaf bags	4 leave bags
Invaded reach:		
***A. mearnsii* (HFA)**	4 leaf bags	4 leave bags
Fynbos species	4 leaf bags	4 leave bags
	macroinvertebrate	
Near pristine reach:		
Fynbos species (HFA)	4 leaf bags	4 leaf bags
A. mearnsii	4 leaf bags	4 leaf bags
Invaded reach:		
***A. mearnsii* (HFA)**	4 leaf bags	4 leaf bags
Fynbos species	4 leaf bags	4 leaf bags

The Hierarchical Framework for Stream Habitat Classification by Frissel et al., (1986) was followed to place both experiment's leaf bags. Microhabitat subsystems are systems defined by Frissel et al., (1986) as patches within pool/riffle systems which have homogeneous environmental conditions (temperature, discharge, substrate characterization). Decomposition and macroinvertebrate leaf bags were placed in sections where microhabitat subsystems were detected within pool systems (Frissell et al., 1986). Mediterranean-climate streams are categorised by successive floods and droughts that are variable through seasons due to high annual and inter-annual discharge (Gasith and Resh, 1999). Pools are seen as habitats which hold inputs of leaf litter and other debris which accumulates and creates new microhabitats through different seasons (Gasith and Resh, 1999; Bonada et al., 2007). The macroinverterbrate and decomposition bags, were placed in pool habitats as these sections of a stream are

generally less affected by flow variability and major changes in synergistic factors (water temperature, pH and conductivity, Grab, 2014).

Zip-lock bags was used to collect decomposition and macroinvertebrate leaf bags and then placed into a cooler with ice and eventually transported to the university laboratory. The bags were disassembled, where the remaining leaf material in each bag was carefully washed off and sieved (250 µm) to remove debris and invertebrates. Material at each incubation day over the study period of decomposition were placed in paper bags with labels dried at 50 °C for 24 h to attain a constant dry mass (g). Sometimes mineral deposits are not washed off the leaves resulting in incorrect final dry mass. This is solved by converting dry mass of each leaf pack to ash-free dry mass (AFDM). The dry mass of each bag was placed into a muffle furnace at 550 °C as organic matter combust at these temperatures (Webster and Benfield, 1986). The mineral ash was then substracted from the dry mass initially recorded which gives the ash-free dry mass or AFDM of each leaf bag. The AFDM represents the percentage mass loss (%) at each inbucation day over the period. To determine ADFM the following equation was used (Equation 4.1).

% AFDM remaining = Final AFDM/Initial AFDM X 100 (Eq. 4.1)

In order to calculate the decomposition rate over time, the expotential model of (Olson, 1963) was used. Where the percentage mass loss at the start of the experiment was (W_0) and and percentage mass loss at time t was (W_t). The constant k is expressed in unit (day^{-1}) and was calculated for each litter bag at each incubation day (Equation 4.2).

$$W_t = W_0 e^{-kt} \quad \text{(Eq. 4.2)}$$

The invertebrates retained in the experiment were sieved and preserved in 90% ethanol in 50 ml vials for later identification. Macroinvertebrates were identified to genus level with an Olympus SZ compound microscope (Model SZ2-ILST) and using the WRC freshwater invertebrate guides (Day et al., 2002b; de Moor et al., 2002b; de Moor et al., 2003b; Stals and de Moor, 2007). The genera were identified belonging to the functional feeding groups (FFG's) of scraper, deposit feeder and predators and were computed according to their FFG's. (2) The genera of these FFG's were then summed within families. (3) Functional Feeding Groups (FFG's) were assigned using Schael, (2005) and the modified version by Merrit and Cummins (1984). Finally, functional

feeding group abundance was standardized to 1 m^2 according to the available surface area of the mesh bag before calculating abundances of the functional feeding groups. The genera identified, their abundances and which functional feeding groups they fall into are reported Appendix E and F.

4.3.2.1 Environmental parameters

Water temperature (°C) at each treatment of each river was recorded every 30 minutes through the experiment (November 2016 – February 2017) with a HOBO U20L Water Level Logger (Onset Computer Corporation Inc., Pocasset, MA, USA, see Appendix B and C). Instantaneous discharge measurements were made at every sampling event with a hand-held Marsh-McBirney Model 2000 flow meter from Southern Waters CC, a consultancy company. Similarly, stream water pH and electrical conductivity were recorded in the field throughout the experiment (see Appendix D) using a Hanna Multiparameter Waterproof Meter (Model - HI9829) from the University of the Western Cape, Earth Sciences Department.

4.3.2.2. Statistical analysis

The data was analysed using the STATISTICA version 13 software package (Dell Inc., 2015). A two way repeated measures analysis of variance (ANOVA) was used to test percentage of remaining ash-free dry mass (AFDM%) over time followed by a Fisher LSD post-hoc test used for means comparison. Decomposition rates (k day^{-1}) were analyzed using a two way repeated measures analysis of variance (ANOVA) followed by a Fisher LSD post-hoc test used for means comparison, which was represented as mean [± SD] over time in Table 4.4.

4.4. Results

4.4.1. Decomposition rates and mass loss after 102 days in near pristine and alien invaded reaches

Table 4.4: Decomposition rates (k day^{-1}) of litter of two plants species, Fynbos species and *A. mearnsii* in near pristine and invaded reaches over days (102) at the Wit and Du Toit's River. Values are represented as k (day^{-1}) mean [± SD] and HFA indicates species in its home environment. Letter (a, b) donate significant differences, P<0.05.

Site Wit River	**k value (day^{-1}) ± SD**
Near pristine reach	
Fynbos species (HFA)	0.0155 ± 0.0086^{b}
A. mearnsii	0.0241 ± 0.0073^{a}
Invaded reach	
A. mearnsii (HFA)	0.0216 ± 0.0107^{a}
Fynbos species	0.0167 ± 0.0064^{b}
Site Du Toit's River	
Near pristine reach	
Fynbos species (HFA)	0.0154 ± 0.0078^{b}
A. mearnsii	0.0225 ± 0.0041^{a}
Invaded reach	
A. mearnsii (HFA)	0.0277 ± 0.0147^{a}
Fynbos species	0.0191 ± 0.0067^{b}

At the Wit River near pristine reach, from day 48 a significant mass was apparent between Fynbos species (mean = 58. 20%) and *A. mearnsii* (34.77%, p<0.001, Figure 4.2a). Between days 64 – 102, *A. mearnsii* losses the most mass in comparison to Fynbos species (p<0.001). In the Fynbos species in general, mass loss remained constant over days, suggesting no home field effect in the near pristine site. The mass loss over the 102 day incubation period was apparent in mean decomposition rate over time (k day^{-1}) in both species, which was significantly different (p<0.05, Table. 4.4).

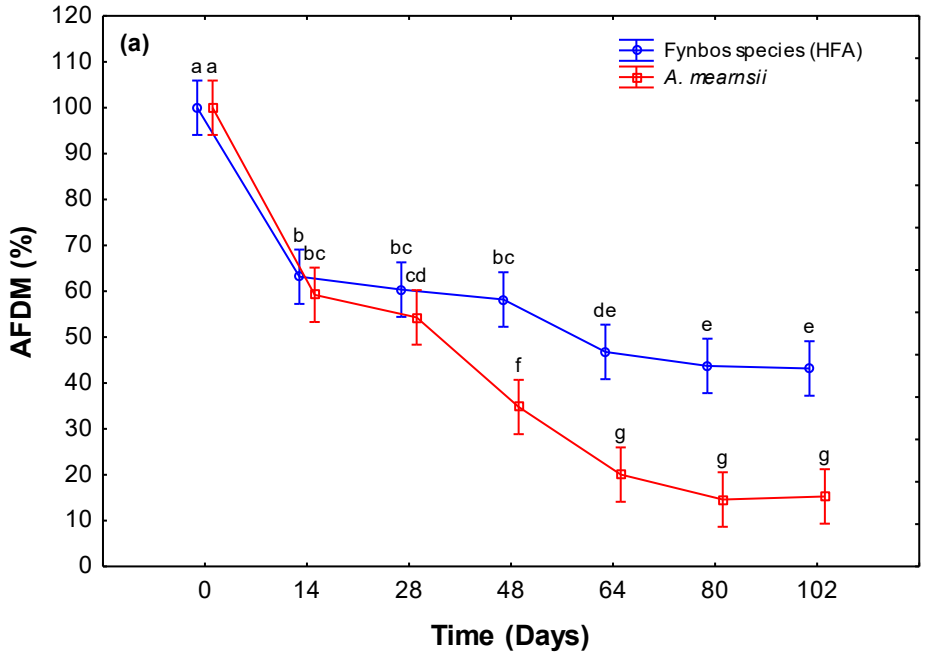

Figure 4.2: Percentage of remaining ash-free dry mass (AFDM) in relation to days of (**a**) Fynbos species (HFA) and *A. mearnsii* at the Wit River, near pristine reach. Letters represent significant differences (LSD test, $p<0.05$) based on a two way repeated measure ANOVA ($F_{[6, 36]} = 9.80$, $p< 0.001$). HFA = indicates species in its home environment.

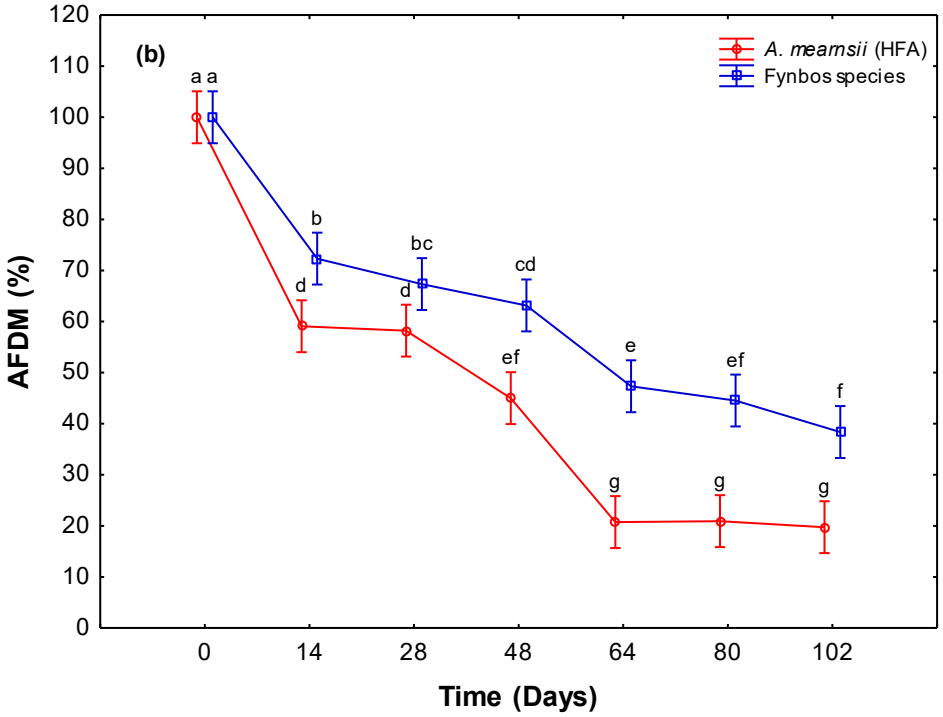

Figure 4.3: Percentage of remaining ash-free dry mass (AFDM) in relation to days of (**b**) *A. mearnsii* (HFA) and Fynbos species at the Wit River, invaded reach. Letters represent significant differences (LSD test, $p<0.05$) based on a two way repeated measure ANOVA ($F_{[6.36]} = 6.52$, $p< 0.001$). HFA = indicates species in its home environment.

In the invaded reach at the Wit River, from day 14 *A. mearnsii* lost significantly more mass than Fynbos species ($F_{[6.36]} = 6.52$, $p<0.001$, Figure 4.3b). The mass loss was at day 64 where *A. mearnsii* lost 20% more mass than Fynbos species ($p<0.001$, Figure 4.3b). *A. mearnsii* in its home environment lost mass much faster than Fynbos species. Overal, *A. mearnsii* decomposition rates (k day^{-1}) show that over time mean values were significantly different between species ($p<0.05$, Table 4.4, invaded reach at the Wit River).

At the Du Toit's, near pristine reach, mass loss of *A. mearnsii* was significantly faster than Fynbos species ($F_{[6.36]} = 9.80$, $p<0.001$, Figure 4.4a). *A. mearnsii* lost leaf mass on average 42.50% more than Fynbos species, which had a mean of 59.34%. The significant mass loss in *A. mearnsii* initially starts from day 48 (mean = 34.77 %) compared to a mean of 58.20% ($p<0.001$, Figure 4.4a) for the Fynbos species. Fynbos species in the near pristine site or 'home site' do not loss substantial mass as *A. mearnsii*, therefore the Home field advantage theory is not apparent. Additionally the decomposition rate of *A. mearnsii* in the site is significantly faster ($p<0.05$) over the fynbos species with a mean of (0.0225 ± 0.0041 k day^{-1}, Table 4.4).

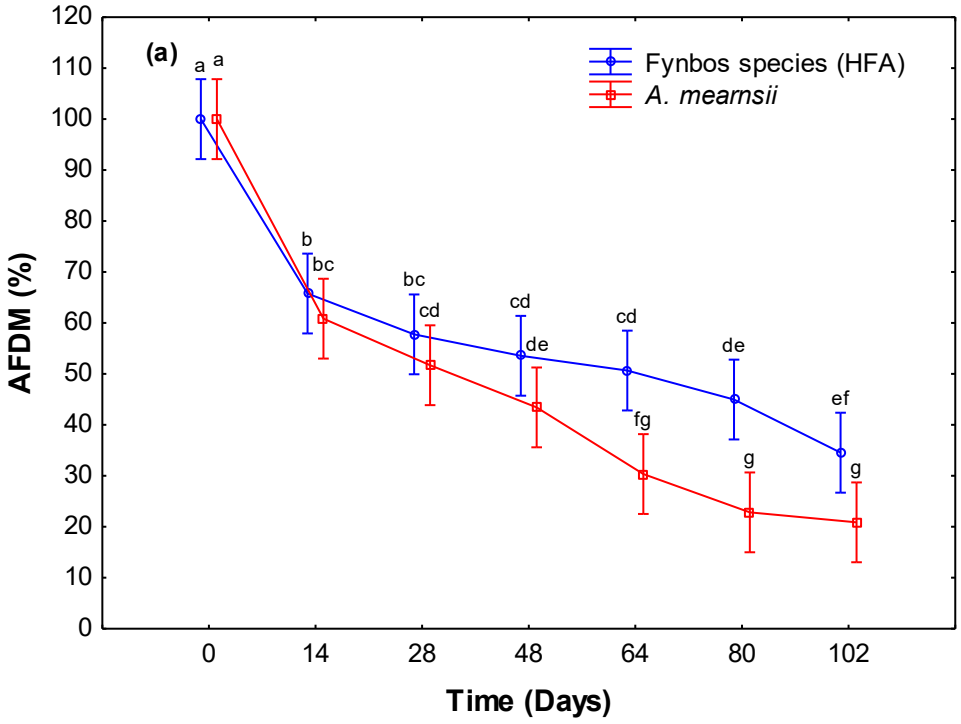

Figure 4.4: Percentage of remaining ash-free dry mass (AFDM) in relation to days of (**a**) Fynbos species (HFA) and *A. mearnsii* at the Du Toit's River, near pristine reach. Letters represent significant differences (LSD test, $p<0.05$) based on a two way repeated measure ANOVA ($F_{[6, 36]} = 2.24$, $p< 0.01$). HFA = indicates species in the home environment.

At the invaded reach, across days, significant differences ($F_{[9.80]} = 9.80$, $p<0.001$, Figure 4.5b) were found between *A. mearnsii* and fynbos species with *A. mearnsii* losing a considerable amount of mass over the incubation period. On days 64 and 80, *A. mearnsii* lost twice as much mass in percentage than the Fynbos species (Figure 4.5b). In table 4.4., decomposition rates between species was significantly different ($p<0.05$) as *A. mearnsii* decomposed at a much faster rate (0.0277 ± 0.0147 k day^{-1}) than Fynbos species (0.0191 ± 0.0067 k day^{-1}).

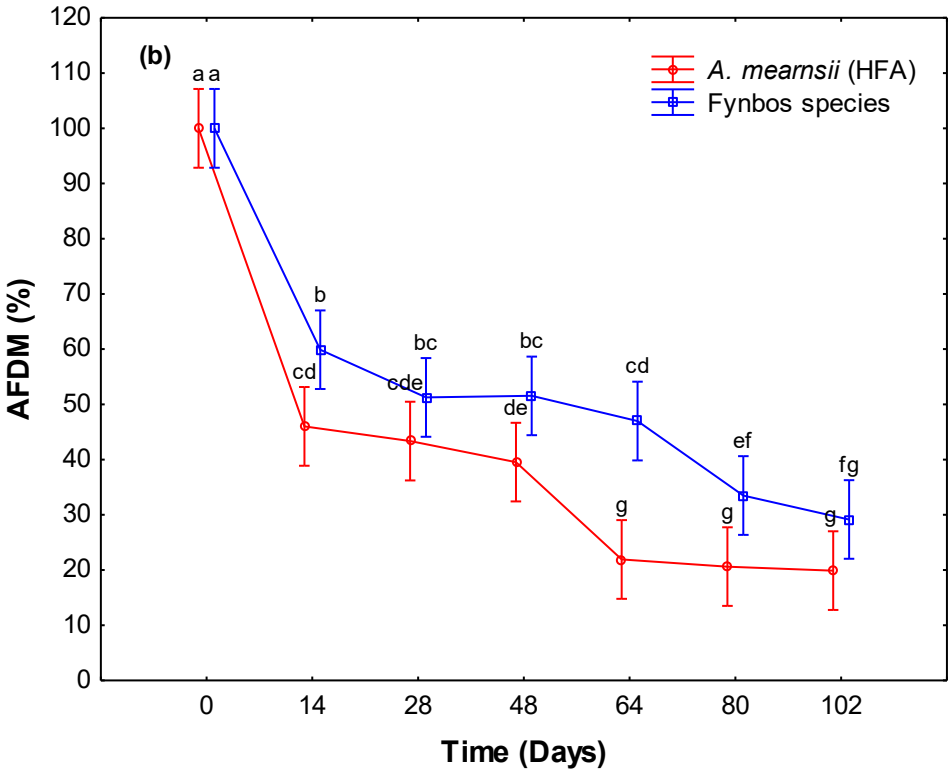

Figure 4.5: Percentage of remaining ash-free dry mass (AFDM) in relation to days of (**b**) *A. mearnsii* (HFA) and Fynbos species Du Toit's River, invaded reach. Letters represent significant differences (LSD test, $p<0.05$) based on a two way repeated measure ANOVA (b) $F_{[6.36]} = 2.31$, $p< 0.01$). HFA = indicates species in its home environment.

4.4.2. Macroinvertebrate community structure on leaf litterbags at near pristine and invaded reaches

4.4.2.1. Macroinvertebrate abundances between near pristine and invaded reaches and general observations

A total of 56 taxa, represented by 1207 macroinvertebrate individuals were sampled across different invasion statuses at both study areas. At the Wit River, 977 macroinvertebrates were sampled at both invasions statuses but only 247 macroinvertebrates at both invasion statuses at the Du Toit's River (Appendix E).

During the sampling events at the Du Toit's River leaf packs were found outside of the stream in some weeks, indicating some human interference, which may have displaced the leaf bags. Leaf bags in the invaded reach at the Du Toit's River also had bags floating on the water, suggesting some flow disturbance, thus some bags had no macroinvertebrates present in leaf packs and were excluded at the time of retrieval.

4.4.3. Functional feeding groups in the litterbags of Fynbos species and *A. mearnsii* leaf litter at different invasion statuses

4.4.3.1. Wit River macroinvertebrate functional feeding group richness

Macroinvertebrate functional feeding groups had no preferences for either Fynbos species litter or *A. mearnsii* leaf litter in the near pristine reach (Figure 4.6). However, functional feeding abundance increased from week 2 until week 12 irrespective if leaf littertype was from its home environment or not. The highest mean in FFG was recorded in *A. mearnsii* leaf litterbags with a mean of 222.67 ± 285.25 m^{-2} (Figure 4.6, Table 4.5) with scrapers accounting for most of the FFG means. Additionally in the Fynbos species leaf litterbags the highest mean FFG was recorded in week 12 (175.00 ± 117.64 m^{-2}) with the functional feeding groups of deposit feeder and predator accounting for the mean values.

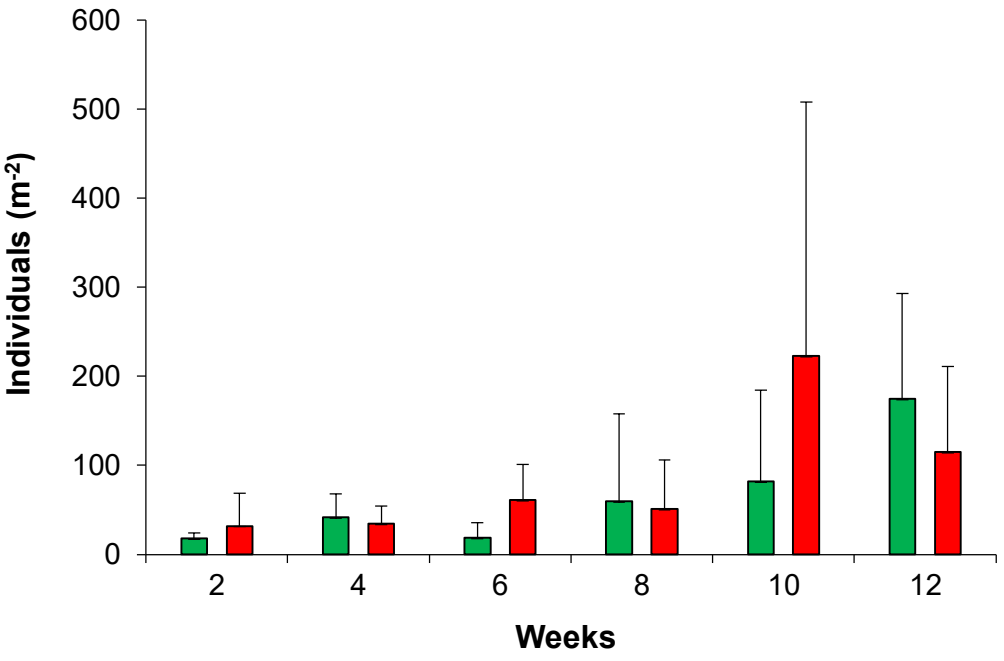

Figure 4.6: Abundance of functional feeding groups of deposit feeder, scrapers and predators (m^{-2}, n = 3) between two leaf litter types (green =Fynbos species (HFA); red = *A. mearnsii*) in the near pristine site at the Wit River. Four leaf packs were sampled of each FFG at every incubation period. HFA = indicates the littertype in its home environment and values are represented as mean [± SD].

Table 4.5: Abundances (mean individuals m^{-2}, ± SD, n = 3) of all functional feeding groups (deposit feeder, scraper and predators) recorded at the Wit River near pristine reach over incubation weeks and different leaf litter types (FS = Fynbos species; AM = *A. mearnsii*). HFA = indicates the littertype in its home environment, (*) indicates a sample size less than 4 and (X) no individuals encountered.

Wit River: Near pristine												
Weeks:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*
Functional Feeding Groups (m^{-2}):												
Deposit Feeder [SD]:	12.00 [6.93]	24.00*	124.50 [26.57]	32.00 [6.93]*	32.00 [18.33]	56.00 [6.93]*	**X**	18. 00 [18.00]	48.00 [21.91]	101.50 [26.35]	196.75 [79.81]	155.25 [46.02]
Scraper [SD]:	24.00 [9.80]	**X**	59.75 [12.6.93]	55.67 [18.06]	24.00 [12.00]	103.67 [6.64]	173.00 [22.72]	89.80 [50.07]	197.00 [60.30]	548.50 [135.96]	48.00 [9.80]	184.75 [64.65]
Predator [SD]:	18 [18.00]*	72.00*	18 [18.00]*	16.00 [13.86]*	**X**	24.00 [12.00]	6.00 [6.00]	**X**	**X**	72.00*	280.25 [107.21]	6.00*
Total Functional feeding groups per litter type	54.00	72.00	125.50	103.70	56.00	183.67	179.00	119.80	245.00	668.00	525.00	346.00
Mean functional feeding groups [SD]	**18.00 [6.00]**	**32.00 [36.66]**	**41.83 [26.01]**	**34.56 [19.96]**	**18.67 [16.65]**	**61.22 [40.09]**	**59.67 [98.20]**	**50.90 [55.01]**	**81.67 [102.72]**	**222.67 [285.25]**	**175.00 [117.64]**	**115.33 [95.83]**

In the invaded reach at the Wit River, similar to the near pristine site, there was no preference for either *A. mearnsii* in its native environment (HFA) or Fynbos species leaf litter in the leaf bags by macroinvertebrate functional feeding groups (Figure 4.7). Functional feeding groups, however increased from week 2 unti 12 with the highest mean values found in the *A. mearnsii* leaf litterbags in week 10 (202. 83 m^{-2}) and in week 12 (184.88 m^{-2}). Scrapers (week 10) and deposit feeders (week 12) accounted for the high values observed (Table 4.6).

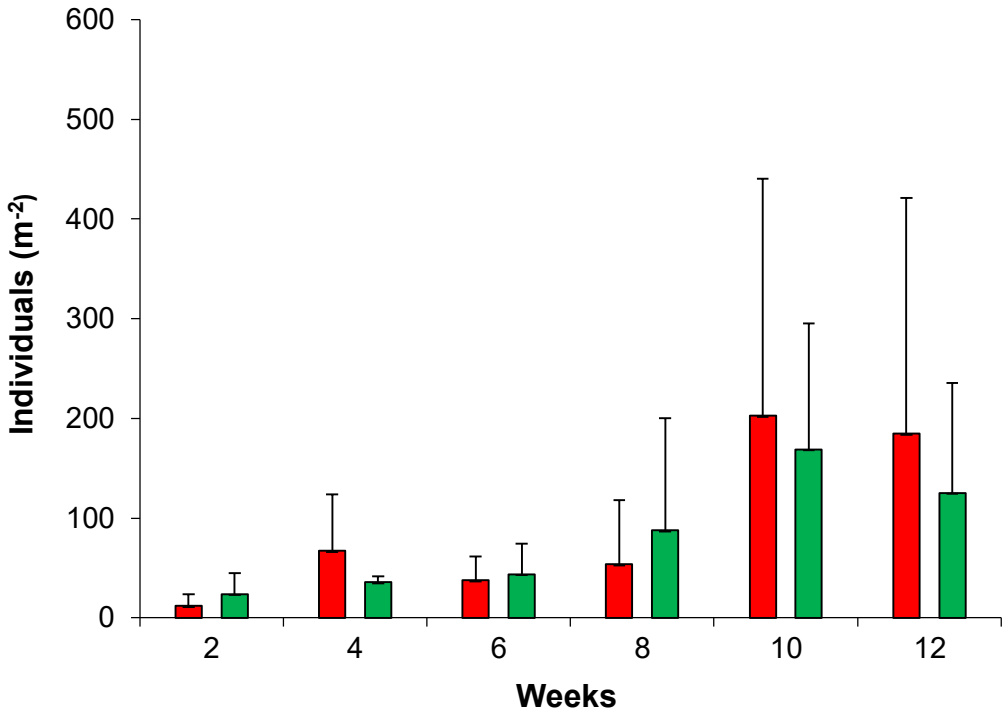

Figure 4.7: Abundance of functional feeding groups of deposit feeder, scrapers and predators (m^{-2}, n = 3 between two leaf litter types (red = *A. mearnsii* (HFA); green =Fynbos species) in the invaded reach at the Wit River. Four leaf packs were sampled of each FFG at every incubation period. HFA = indicates the littertype in its home environment and values are represented as mean [± SD].

Table 4.6: Abundances (mean individuals m^{-2}, ± SD, n = 3) of all functional feeding groups (deposit feeder, scraper and predators) recorded at the Wit River invaded reach over incubation weeks and different leaf litter types (FS = Fynbos species; AM = *A. mearnsii*). HFA = indicates the littertype in its home environment, (*) indicates a sample size less than four and (X) no individuals encountered.

Wit River: Invaded site												
Weeks:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS
Functional Feeding Groups (m^{-2}):												
Deposit Feeder [SD]:	12.00 [6.93]	48.00 [16.97]	53.75 [14.87]	41.50 [14.77]	59.00 [6.35]	77.50 [26.35]	35.50 [11.50]	18. 00 [18.00]	119.25 [68.12]	256.25 [22.83]	361. 75 [91.25]	232.75 [39.54]
Scraper [SD]:	**X**	12.00 [8.49]*	65.50 [40.57]	35.75 [22.76]	41.75 [17.75]	35.50 [20.50]	125.25 [32.85]	89.80 [50.07]	471.25 [226.86]	226.75 [96.18]	351.75 [177.04]	131.25 [58.74]
Predator [SD]:	24.00 [16.97]	12.00 [8.49]*	12.00 [6.93]*	30.00 [18.00]*	12.00*	18.00 [6.00]	**X**	**X**	215.00	24.00 [16.97]	18.00 [6.93]	12.00*
Total Functional feeding groups per litter type	36.00	72.00	202.00	107.25	112.75	131.00	160.75	119.80	608.50	507.00	731. 50	376.00
Mean functional feeding groups [SD]:	**12.00 [12.00]**	**24.00 [20.78]**	**67.33 [56.27]**	**35.75 [5.75]**	**37.58 [23.78]**	**43.67 [30.58]**	**53.58 [64.55]**	**87.67 [112.86]**	**202.83 [237.90]**	**169.00 [126.44]**	**184.88 [236.00]**	**125.33 [110.49]**

4.4.3.2. Du Toit's River macroinvertebrate functional feeding group richness

The Du Toit's River, in the near pristine reach showed no preference from functional feedings groups for either Fynbos species leaf litter, which was in its native environment (HFA) or *A. mearnsii* litter (Figure 4.8, Table 4.7). The highest mean value found across weeks was in *A. mearnsii* leaf litterbags in week 12, which had a mean value of 167.00 m^{-2}. In week 10, *A. mearnsii* litter showed no FFG individuals encountered as all bags sampled of the respective littertype went missing. Hence, no data was recorded.

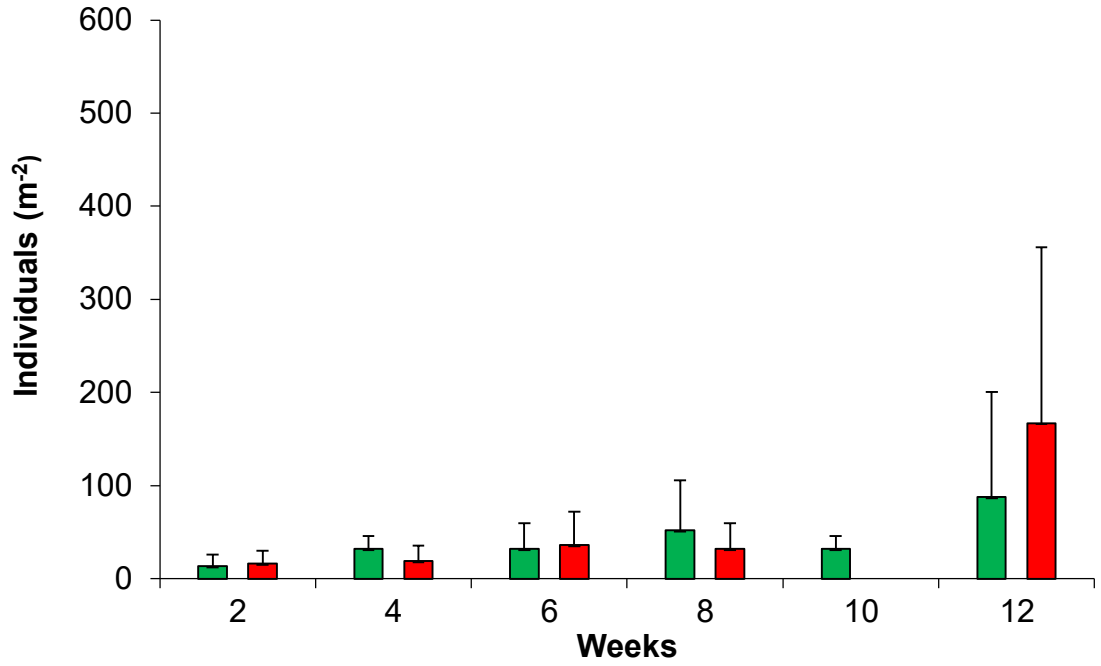

Figure 4.8: Abundance of functional feeding groups of deposit feeder, scrapers and predators (m^{-2}, n = 3 between two leaf litter types (green =Fynbos species (HFA); red = *A. mearnsii*) in the near pristine reach at the Du Toit's River. Four leaf packs were sampled of each FFG at every incubation period. HFA = indicates the littertype in its home environment and values are represented as mean [± SD].

Table 4.7: Abundances (mean individuals m^{-2}, ± SD, n = 3) of all functional feeding groups (deposit feeder, scraper and predators) recorded at the Du Toit's River near pristine reach over incubation weeks and different leaf litter types (FS = Fynbos species; AM = *A. mearnsii*). HFA = indicates the littertype in its home environment, (*) indicates a sample size less than four and (X) no individuals encountered.

Du Toit's River: near pristine												
Weeks::	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*	FS(HFA)	*AM*
Functional Feeding Groups (m^2):												
Deposit Feeder [SD]:	24.00 [0.00]	24.00*	48.00*	**X**	48.00*	**X**	**X**	**X**	24.00*	**X**	215.00*	381.5 [119.50]*
Scraper [SD]:	**X**	**X**	24.00*	32.00 [6.93]	**X**	36.00 [12.00]	107.50 [35.50]	48.00*	24.00*	**X**	48.00*	95.50 [23.50]*
Predator [SD]:	16.00 [9.80]	24.00	24.00*	24.00*	48.00 [24.00]	72.00*	48.00*	48.00*	48.00*	**X**	**X**	24.00*
Total Functional feeding groups per litter type	40.00	40.00	96.00*	56.00	96.00	108.00	155.50	96.00	96.00	X	263.00	501.00
Mean functional feeding groups [SD]	**13.33 [12.22]**	**16.00 [13.86]**	**32.00 [13.86]**	**18.67 [16.65]**	**32.00 [27.71]**	**36.00 [36.00]**	**51.83 [53.85]**	**32.00 [27.71]**	**32.00 [13.86]**	**X**	**87.67 [112.86]**	**167.00 [189.17]**

Functional feeding groups in the invaded reach at the Du Toit's River showed no liking for either *A. mearnsii* litter or Fynbos species leaf litter in the reach (Figure 4.9, Table 4.8). Threre was also no general increase in FFG across week as this was also apparent at the near pristine site at the Du Toit's River. Functional feedings groups was only higher than 100 m^{-2} in one leaf littertype, which was the Fynbos species litter 143.31 ± 48.95 m^{-2} in week 12. Macroinvertebrate abundances were low and variable at the each respective reach at the Du Toit's River.

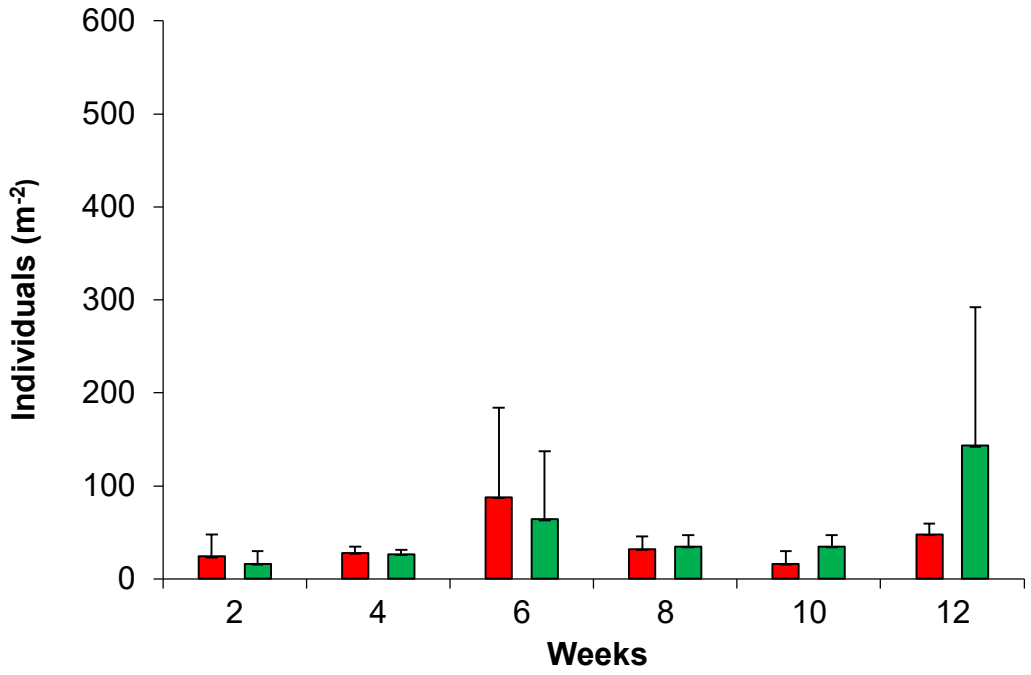

Figure 4.9: Abundance of functional feeding groups of deposit feeder, scrapers and predators (m^{-2}, n = 3 between two leaf litter types (red = *A. mearnsii* (HFA); green = Fynbos species) in the invaded reach at the Du Toit's River. Four leaf packs were sampled of each FFG at every incubation period. HFA = indicates the littertype in its home environment and values are represented as mean [± SD].

Table 4.8: Abundances (mean individuals m^{-2}, ± SD, n = 3) of all functional feeding groups (deposit feeder, scraper and predators) recorded at the Du Toit's River near pristine reach over incubation weeks and different leaf litter types (FS = Fynbos species; AM = *A. mearnsii*). HFA = indicates the littertype in its home environment, (*) indicates a sample size less than four and (X) no individuals encountered.

Du Toit's River: Invaded site												
Weeks:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS	*AM*(HFA)	FS
Functional Feeding Groups (m^{-2}):												
Deposit Feeder [S.D]:	48.00*	24.00*	36.00 [12.00]*	24.00*	**X**	**X**	24.00 *	32.00 [6.93]	24.00*	32.00 [8.00]	48.00*	310.25 [76.05]
Scraper [S.D]:	**X**	**X**	24.00*	32.00 [6.93]	191.00 [39.67]	144.00	48.00*	47.75 [16.74]	24.00*	47.75 [16.74]	36.00 [12.00]	95.67 [56.04]
Predator [S.D]:	24.00*	24.00*	24.00*	24.00*	72.00 [8.00]	48.00	24.00*	24.00*	**X**	24.00*	59.50 [35.50]	24.00*
Total Functional feeding groups per litter type	72.00	48.00	84.00	80.00	263.00	192.00	96.00	103.00	48.00	103.75	143.50	429.92
Mean functional feeding groups [S.D]	**24.00 [24.00]**	**16.00 [13.86]**	**28.00 [6.93]**	**26.67 [4.62]**	**87.67 [96.46]**	**64.00 [73.32]**	**32.00 [13.86]**	**34.58 [12.08]**	**16.00 [13.86]**	**34.58 [12.08]**	**47.83 [11.75]**	**143.31 [148.95]**

4.4.3.3. Distributions of macroinvertebrate genera in the litterbags of Fynbos species and *A. mearnsii* in different invasion statuses at the Wit River

The number of genera individuals sampled at the Wit River, in the near pristine reach were low and variable, with the minimum mean recorded for *Castanophlebia* and *Paramerina* 23.00 ± 0.00 m^{-2} both in *A. mearnsii* leaf litterbags (Figure 4.10, Table 4.9). The individual's that was the most encountered at the near pristine site was *Strina aequalis* on both leaf littertypes, with *A. mearnsii* being the highest recorded (Table 4.9).

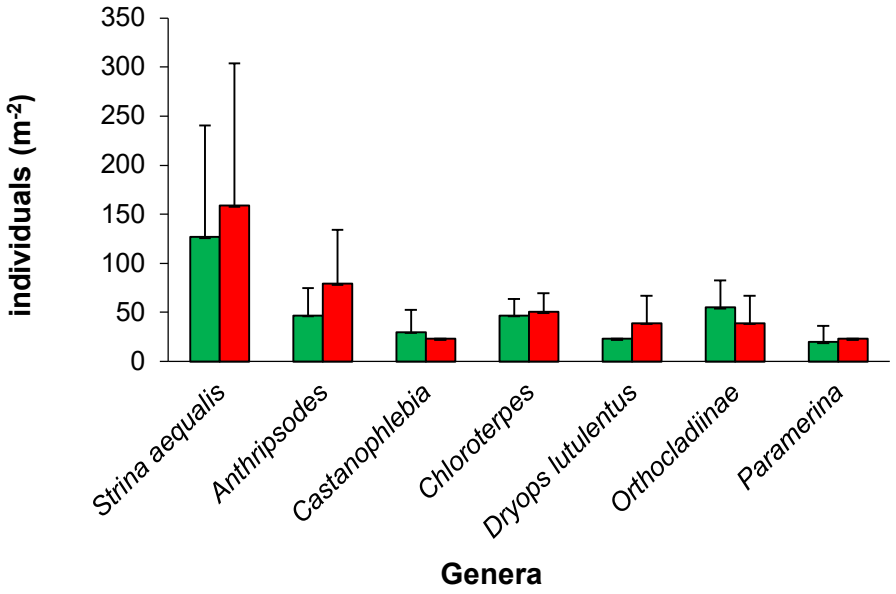

Figure4.10: Abundances of the major genera (individual's m^{-2}, n=7) sampled across incubation weeks (12) between two leaf littertypes (green = Fynbos species (HFA); red =*A. mearnsii*) in the near pristine reach at the Wit River. HFA = indicates the littertype in its home environment and values are represented as mean [± SD].

Table 4.9: Abundances of the major genera (individuals m^{-2}, n=7) and their associated functional feeding groups sampled across 12 incubation weeks between two leaf littertypes of Fynbos species (HFA) and *A. mearnsii* in the near pristine reach at the Wit River. Values are represented as mean [± SD] and HFA indicates the littertype in its home environment.

Reach: Near pristine			
Genera	Functional feeding groups (FFG)	Fynbos species littertype (HFA) mean ± [SD]	*A.mearnsii* littertype mean ± [SD]
Strina aequalis	scraper	126.78 ± 114.07	158.87 ± 145.08
Anthripsodes	deposit feeder	47.00 ± 27.71	79.00 ± 54.99
Castanophlebia	depost feeder	30.00 ± 22.61	23.00 ± 0.00
Chloroterpes	predator	47.00 ± 16.97	50.33 ± 19.34
Dryops lutulentus	srapper	23.00 ± 0.00	39.00 ± 27.71
Orthocladiinae	scraper	55.00 ± 27.71	39.00 ± 27.71
Paramerina	predator	19.86 ± 16.15	23.00 ± 0.00

At the Wit River, in the invaded reach, the mean number of individuals were generally constant for most genera individuals encountered (Figure 4.11, Table 4.10). The highest mean recorded was *Strina aequalis* in the Fynbos species leaf lttertype (140. 36 m^{-2}) with a similar range in mean value (130.04 m^{-2}) also recorded in *A. mearnsii* leaf litterbags in the invaded reach across weeks (Table 4.10).

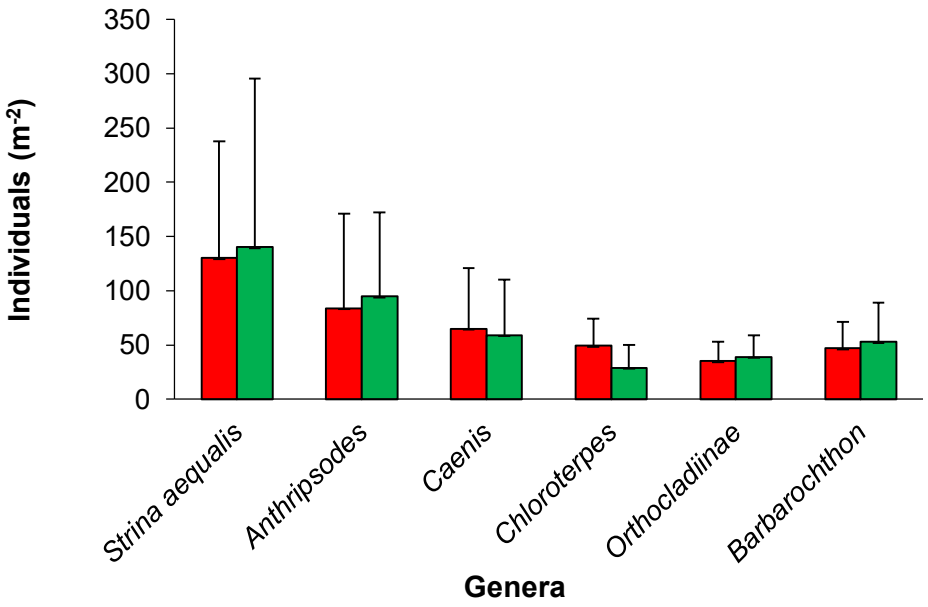

Figure 4.11: Abundances of the major Genera (individuals m^{-2}, n=6) sampled across incubation weeks (12) between two leaf littertypes (red = *A. mearnsii* (HFA); green = Fynbos species) in the invaded reach at the Wit River. HFA = indicates the littertype in its home environment and data is reflected in mean [± SD].

Table 4.10: Abundance of the major Genera (individuals m^{-2}, n=6) and their associated functional feeding groups sampled across 12 incubation weeks between two leaf littertypes of *A. mearnsii* (HFA) and Fynbos species in the invaded reach at the Wit River. Values are represented as mean [± SD] and HFA indicates the littertype in its home environment.

Reach: Invaded			
Genera	Functional feeding groups (FFG)	*A.mearnsii* littertype (HFA) mean ± [SD]	Fynbos species littertype mean ± [SD]
Strina aequalis	scraper	130.06 ± 107.86	140. 36 ± 154.86
Anthripsodes	deposit feeder	83.95 ± 87.15	94.86 ± 77.32
Castanophlebia	depost feeder	65.00 ± 55.00	59.00 ± 50.91
Chloroterpes	predator	49. 18 ± 25.07	29.00 ± 20.78
Dryops lutulentus	scraper	35.00 ± 18.14	39.00 ± 19.60
Orthocladiinae	scraper	47.00 ± 24.00	53.00 ± 36.00

4.4.3.4. Distributions of macroinvertebrate genera from the litterbags of Fynbos species and *A. mearnsii* littertypes in different invasion statuses at the Du Toit's River

The near pristine reach at the Du Toit's River, the highest genera of *Caenis* appeared on *A. mearnsii* leaf litter across the sampling period with a mean of 64.91 m^{-2} (Figure 4.12, Table 4.11). In addition, genera was observed to be generally low in mean values for other species *(*Figure 4.12).

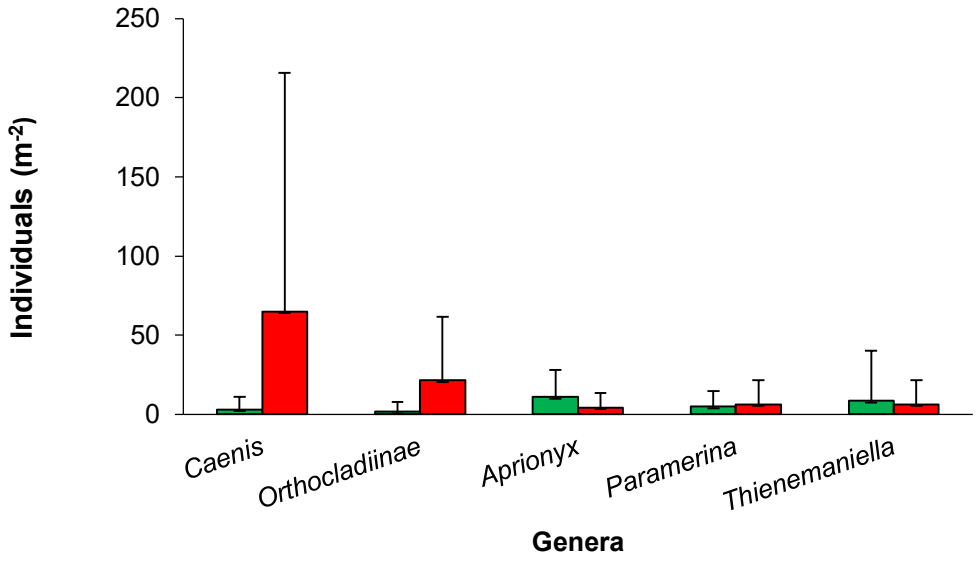

Figure 4.12: Abundances of the major Genera (individuals m^{-2}, n=5) sampled across incubation weeks (12) between two leaf littertypes (green = Fynbos species (HFA); red = *A. mearnsii*) in the near pristine reach at the Du Toit's River. HFA = indicates the littertype in its home environment and Values are represented as mean [± SD].

Table 4.11: Abundance of the major Genera (individuals m^{-2}, n=5) and their associated functional feeding groups sampled across 12 incubation weeks between two leaf littertypes of Fynbos species (HFA) and *A. mearnsii* in the near pristine reach at the Du Toit's River. Values are represented as mean [± SD] and HFA indicates the littertype in its home environment.

Reach: Invaded			
Genera	Functional feeding groups (FFG)	Fynbos species littertype (HFA) mean ± [SD]	*A.mearnsii* littertype mean ± [SD]
Caenis	deposit feeder	3.07 ± 8.09	64.91 ± 150.83
Orthocladiinae	scraper	1.64 ± 6.15	21.45 ± 40.26
Aprionyx	deposit feeder	10.87 ± 17.39	4.18 ± 9.30
Paramerina	predator	4.93 ± 9.79	6.36 ± 15.14
Thienemaniella	scraper	8.50 ± 31.80	6.36 ± 15.14

In the invaded site the genera the most frequently encountered over the weeks was *Caenis* which had a mean of 54.92 m^{-2} but specifically in *A. mearnsii* leaf litterbags (Figure 4.13, Table 4.12). Furthermore, the genera of *Thienemaniella*, *Anthripsodes* and *Orthocladiinae* had similar mean values across weeks on each respective leaf litterbag.

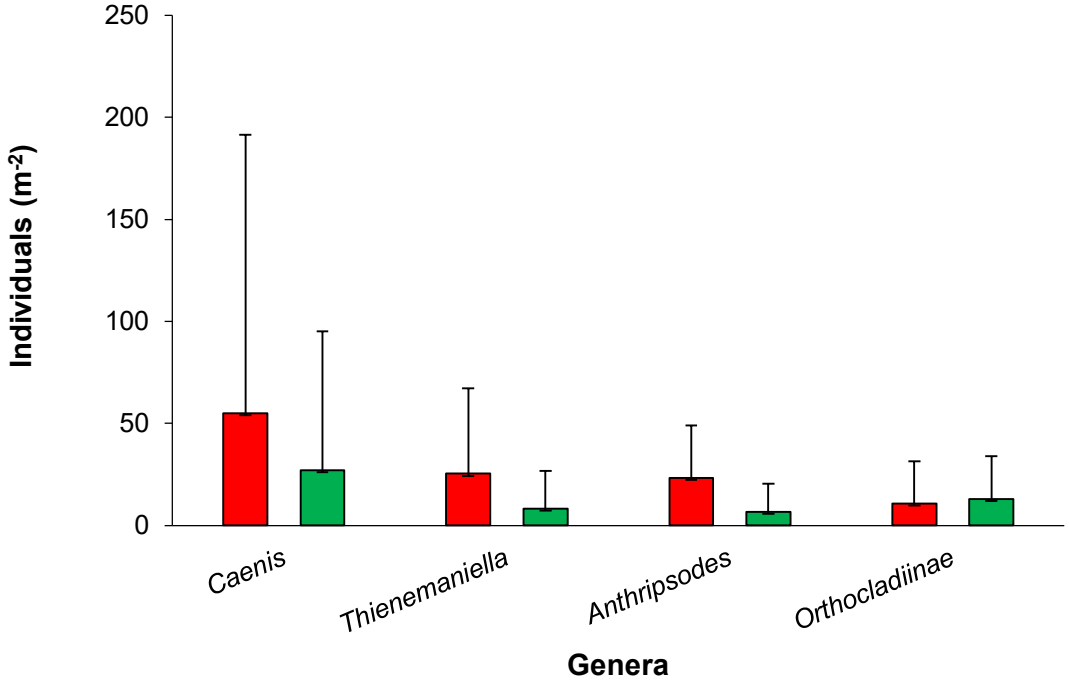

Figure 4.13: Abundances of the major Genera (individual's m^{-2}, n=4) sampled across incubation weeks (12) between two leaf littertypes (red = *A. mearnsii* (HFA); green = Fynbos species) in the invaded reach at the Du Toit's River. HFA = indicates the littertype in its home environment and data is reflected in mean [± SD].

Table 4.12: Abundance of the major Genera (individuals m^{-2}, n=4) and their associated functional feeding groups sampled across 12 incubation weeks between two leaf littertypes of *A. mearnsii* (HFA) and Fynbos species in the invaded reach at the Du Toit's River. Data is reflected in mean [± SD] and HFA indicates the littertype in its home environment.

Reach: Invaded			
Genera	Functional feeding groups (FFG)	*A.mearnsii* littertype (HFA) mean [± SD]	Fynbos species littertype mean [± SD]
Caenis	deposit feeder	54.92 ± 136.37	27.06 ± 68.19
Thienemaniella	deposit feeder	25.38 ± 41.74	8.29 ± 18.47
Anthripsodes	depost feeder	23.46 ± 25.46	6.82 ± 13.70
Orthocladiinae	predator	10.77 ± 20.66	13.13 ± 20.99

4.5. Discussion

4.5.1. Decomposition rates between Fynbos species and *A. mearnsii* in near pristine and invaded reaches

The results supported previous findings of invasive plants decomposing at a faster rate than native species (Witkowski, 1991a; Allison and Vitousek, 2004; Kueffer et al., 2008; Claeson et al., 2014). These results indicate that *A. mearnsii* litter is decomposed more rapidly than native species litter, regardless of the local environment or the invasion status. Allison and Vitousek, (2004) found in the nutrient poor ecosystems of Hawaiian Islands that invasive species decomposed faster than the native species. In the nutrient impoverished ecosystem of the granitic Seychelles, Kueffer et al., (2008) found in a field experiment between six native and six invasive plant species that the invasives decomposed at a much faster rate. In addition, in the Western Cape (Fynbos biome), Witkowski, (1991a) found that the decomposition rate of *A. saligna* was faster than the native sclerophyllous shrub *Leucospermum parile*.

Witkowski, (1991a); Allison and Vitousek, (2004); Bengtsson et al., (2012) and Hickman et al., (2013) all suggests that the variation in decomposition rates are largerly attributed to difference in species ecological traits, such as different plant strategies and nutrient composition.

The outcomes from the present study supports this conclusion, as we found faster decomposition rates at the Wit and Du Toit's River in both reaches. Jo et al., (2016) found that decomposition rates over time were related to high N concentrations in litter. In the present study, the litter from the invasive N_2-fixing *A. mearnsii* was considerably different in litter quality (high mean litter N concentrations and lower mean litter C:N ratios (see section 3.5.1, Table 3.2) than the fynbos species. The differences in litter quality between native and invasive species have been shown in other studies (Santiago, 2007; Gießelmann et al., 2011; Prescott et al., 2016) as the main factor in faster decomposition rates for invasive species over time. The slow decomposition rates in the study was found by others in the Fynbos biome for native species (Mitchell et al., 1986; Witkowski, 1991a; Stock and Allsopp, 1992) which is mainly attributed to high C:N ratios. Recently, Bengtsson et al., (2011) further supported these finding by stating that C:N ratios of leaf litter is good indicator of decomposition rates in the Fynbos biome (or renosterveld), although large difference in litter decay rates can be found in the Fynbos biome as there are a hyperdiversity of plant species found in the region (Bengtsson et al., 2011; 2012). In the present study climate as a factor can be excluded as invasion status per river are reasonably close to one another, and the exclusion of climate was noted bt others in their studies (Meentemeyer, 1978; Gholz et al., 2000). Therefore, the major contributing factor of decay rates of *A. mearnsii* in both near pristine and invaded reaches at both rivers are likely related to

intrinsic variables of leaf litter biochemistry (Perez-Harguindeguy et al., 2000; Ehrenfeld, 2003; Liao et al., 2008). However, this may not be the only reason for faster decomposition rates.

Generally, decomposition trials demonstrate that within a river hierarchy (upstream and downstream) breakdown of leaf litter is different on a site level (Ayrez, 1997; Sponseller and Benfield, 2001; LeRoy et al., 2006). This is mainly due to these sections having unique instream characteristics such as water temperature, flow regime, pH, conductivity and microhabitats (Frissel et al., 1986; LeRoy et al., 2006). Stream characteristics between near pristine and invaded reaches were similar at the Wit River. Stream environmental factors of average temperature, discharge, pH and EC show little variation in change through the study period at the Wit River reaches. At the Du Toit's River, a similar pattern in stream characteristics between pristine and invaded reaches in pH and conductivity (EC) were observed. However, the range of discharge rates (m^3 s^{-1}) between near pristine (0.16 to 0.40 m^3 s^{-1}) and invaded reaches (0.43 to 0.71 m^3 s^{-1}) are different and could account for slower decay rates in the near prestine reach at Du Toit's River during the incubation period. In a study by Claeson et al., (2014) in the Chehalis River basin, Washington USA, it was found that decay rates were affected by physical abrasion of leaf litter at the intermediate stage of decay as stream flow ranged from winter low flows to irregular bank-full flow events. Therefore, physical abrasion due to the discharge regime between sites could account for the slower and faster decay rates. Mediterranean climate streams are characterized by high inter-annual and intra-annual variability in discharge, which can play a role in decay rates (Gasith and Resh, 1999). In addition, Graça et al., (2010) stated the discharge regime across different environmental gradients needs to be considered when predicting litter decay rates. Moreover, temperature seems to play a role in the retarding decay rates in near pristine sites. Between sites at the Du Toit's River, mean water temperature differed with 1.58˚C, with the near pristine site being colder over the incubation period. Braatne et al., (2007) in a study on Japanese knotweed along riparian corridors in Clearwater River Basin, USA found that lower temperatures decreased decomposition rates in the intermediate stage for all leaf types measured in their study. Another factor to take account in the experiment is litterbag size. In the study nylon fine mesh litter bags (0.5 mm) were used to exclude meso- and macro-invertebrate fauna (Webster and Benfield, 1986). Thus, the assumption drawn from the decomposition study is that there was minimal interaction between macroinvertebrates and leaf litter. Therefore, results found in the decomposition rates in the study are not influenced by macroinvertebrate communities, but rather bacterial/fungal communities as noted by Graça et al., (2001).

Different plant species leaf litter decomposes at different rates over time, which attributed to difference in leaf traits within a stream and can account for up to 70% of the disparity explained in decay processes (Hickman et al., 2013; Kuglerova et al., 2017). The other ± 30% is

explained by factors such as stream characteristics (LeRoy et al., 2006) and HFA effects (Gholz et al., 2000). In the study at both rivers reaches (near pristine and invaded) decomposition rates are effected by plant quality and therefore viewed as holistic comprehensive metric for decay rates between *A. mearnsii* and fynbos species. The reaches in the study are relatively similar (only the near pristine site in Du Toit's River has a lower discharge) and invasion statuses are largely close to each other, which show less heterogeny in stream characteristics. Addtionally, there is no home field effect of litter decomposing faster in its home environment as stated by others (Gholz et al., 2009; Austin et al., 2014), instead it was only related to leaf nutrient composition between species. The present study is one of the first to compare decomposition rates in stream between native and invasive plants in the Fynbos biome. In addition, it adds to the growing knowledge of leaf litter processing in aquatic environments as it is not as well documented as terrestrial decomposition studies (Witkowski, 1991a; Allison and Vitousek, 2004). *A. mearnsii* decompose faster in unexploited areas (e.g near pristine sites). Consequently, ecosystem function in "undisturbed" streams may be affected by *A mearnsii* as the invasion of the species can increase the amount of leaf material (litter with high N and P) entering (see chapter 3, section 3.6.1 and 3.6.3 resorption proficiencies) the stream and fast cycling of N and P during decomposition. The Fynbos biome is known for its species diversity of aquatic biota, particularly invertebrate communities (de Moor and Day, 2013). Therefore, with decomposition of the nutrient rich leaves of the invasive species can likely alter aquatic environments.

4.5.2. Macroinvertebrate litter affinity effects in home and away environments in *A. mearnsii* and fynbos species

There was no general preference of functional feeding groups or of genera diversity and abundance for either Fynbos species or *A. mearnsii* leaf litter regardless of the local environment or in the study sites at each invasion status. In early weeks at the Wit River at both reaches, there was no increase in functional feeding groups for both leaf littertypes. However, from week 8 to 12 a general increase is apparent whether litter was from its home environment or introduced. The taxa richness and diversity of macroinvertebrates coincided with changes in the quantities of litter inputs and the seasonal timing of inputs. This was evident early in the summer month of December for both native species and *A. mearnsii* (see Chapter 3, section 3.6.1). It is therefore likely that the macroinvertebrate communities are seasonally dependent on the pulse of leaves entering the stream in early summer. Similar results are found in riparian zones in the USA by Reinhart and VandeVoort, (2006) and Claeson et al., (2014) and where sites received a diverse input of litter types, which resulted in an upturn in invertebrate communities. *Strina aequalis,* which belong to functional feeding group of scrapers, accounted for most of the abundances across weeks and showed a preference for both fynbos species and *A. mearnsii*

leaf littertype. This suggests that the leaf litter quality (invasive vs. native) regulated the abundance of the species across weeks. In a laboratory experiment, which resembled natural conditions by Januskauskaite and Straige, (2011), found that leaf litter inputs regulated the abundances of the organisms in their study. The finding also suggests that introduction of *A. mearnsii* litter may effectively be utilized by *Strina aequalis* as a potential energy source. In addition, the near pristine site at the Wit River is constituted of large substratum of material facilitating scavenging of macroinvertebrate communitites. The rough surface topography (see section 4.3.1) creates microhabitats, accompanied with seasonal leaf litter inputs, which facilitates macroinvertebrate community diversity in stream and was found in other studies (Holomuzki and Messier, 1993; Rempel et al., 2000). In the invaded reach however, functional feeding groups recorded was greater suggesting that the resources (food or habitat availability) *A. mearnsii* invasion sites may hold are crucial to these groups, as was noted by Samways et al., (2011). Reinhart and VandeVoort, (2006) in their study in the USA indicated that macroinvertebrate community's reaction and change was inevitable when 50% of the knotweed species invaded the riparian areas which may hold true for the invaded reach at the Wit River.

At the Du Toit's River in both reaches, there was no general preference from functional feeding groups to the littertypes or interms of abundances of genera distributions. In the near pristine reach, the low number of functional feeding groups was associated to stream characteristics and human interference. The site is situated in the mountain stream section of the Du Toit's River, which has a high gradient and narrow channels, a physical feature of the Franschhoek Mountains (Tharme and Brown, 2004). Wall et al., (2008) and Tharme, (2010) sugegsted that steep river slopes and cold-water temperatures can lead to a decline in invertebrate diversity and abundances and this was present in the study reach (section 4.3.1, Table 4.2). Due to human interference, many macroinvertebrate bags (including macorinvertebrates) were lost during the sampling weeks. Bags were placed outside of the stream or displaced from the original sampling point, thus common trends could not be deduced at the Du Toit's, near pristine reach. The invaded reach was situated close to the Theewaterskloof Reservoir. The site was dominated by small cobbles, gravel and sand (fine and coarse) and this type of substratum has been noted by Hussain and Pandit, (2012) as the poorest habitat for macroinvertebrates. In a study by Rempel et al., (2000) in the Frazer River in British Columbia-USA, found that macroinvertebrates did not favour sites with sandy substratums. Generally sites with sandy substratums are associated with high silt fractions (Rempel et al., 2000). In the same study reach, Wiener, (2018) found that the reach consisted of approximately ± 90% silt fraction. Sandy substratums are associated with high percentage silt fraction and have been found to down regulate macroinvertebrates in South Africa (Dallas and Day 1993; Samways et al., 2011).

Stream dwelling macroinvertebrates are regulated by many factors such as seasonal riparian leaf litter inputs, substratum characteristics and essential resources. The studies of Reinhart and VandeVoort (2006); Tharme, (2010); Samways et al., (2011) and Hussain and Pandit, (2012), correspond to the finding presented here, which shows that the macroinvertebrates in the present study is regulated by the same factors at the different invasion statuses but particularly the invaded reach at Du Toit's River.

However, the discharge regime in Mediterranean streams of the CFR is unpredicatable and is season dependent (Gasith and Resh, 1999). The unpredicatable discharge causes winter floods and summer droughts that cause challenging conditions for aquatic ecosystems (de Moor and Day, 2013). This exerts a selective pressure on communities of macroinvertebrates, to synchronize with certain seasons as food sources become available in stream from the adjacent riparian area (Gasith and Resh, 1999; de Moor and Day, 2013). Many macroinvertebrate communities with short life cycles and small body sizes appear in early summer till early autumn (Maamri et al., 1994), which may be the holistic reason in high abundance of macroinvertebrate species particularly in the Wit River reaches. Furthermore, there was no indication in the study whether macroinvertebrate communities had a liking or 'affinity effect' to their home turf or introduced litter, suggesting that the HFA for macroininvertebrate and decomposition rates might be in its primitive stages in a region like the Fynbos biome riverine systems. The ecological data obtained from one river and study reaches of each cannot be extrapolated to another, with each river and reach having to be assessed individually to seek common trends.

4.6. Conclusion

This study demonstrates that the invasion of *A. mearnsii* in undisturbed environments may have an effect on aquatic systems. The effect of *A. mearnsii* leaf litter decomposition, which is faster than that of native species, mainly due to key chemical traits, may extend beyond the local area of invasion and to other undisturbed environments (Reinhart and VandeVoort, 2006). In the present study the decay rates of the IAP appeared to be same in pristine and invaded environments, which demonstrates the potential of *A. mearnsii* to alter aquatic environments whether close to its invasive reach or further away, even in reaches not directly affected by invasion. This context is well described by Callaway and Ridenour, (2004), which noted that the chemical complexes produced by IAP affects in stream macroinvertebrates in the area they invade as these organisms are not well adapted to these exotic compounds. Understanding how litter quality influences invertebrate communities is important in restoration projects that often overlook the effect invasion of IAP has on communities of macroinvertebrates, especially in aquatic environments. Therefore, it is imperative that we understand how IAP's or the litter quality of the species affects invertebrates, particularly in the Fynbos biome where research on

macroinvertebrates still remain patchy. In addition this will aid to help preserve the rich diversity of invertebrate coummunities of rivers in the Fynbos Biome.

Chapter 5

General conclusions, management implications and future research

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5.1. General conclusions and management implications

The research in the thesis is among the first to address different aspects of plant nutrient dynamics in fynbos riparian zones invaded by *A. mearnsii*, as well as adjacent aquatic environments. Leaf litterfall, litter decay rates and macroinvertbrate assemblages suggests a major impact of this invasive species on riparian environments as well as aquatic environments, though invertebrate communities seem less affected by the invasive species. The motivation for this study was to gain a better understanding of riparian zone function in the mountain streams and mountain transitional zones in the Breede Water Management Area (WMA) in the Western Cape. This chapter presents the major findings, based upon two fynbos mountain streams of the Fynbos biome.

The overall purpose of this study was to quantify and compare the leaf litter rate and N inputs, decomposition rates and macroinvertebrate community assemblages in near pristine (reference sites) and invaded sites (predominantly of *A. mearnsii*) between co-occurring native species (*Brabejum stellatifolium* and *Metrosideros angustifolia*) and the invasive alien species, *Acacia mearnsii*. Seasonal leaf litterfall and nutrient concentration patterns were analyzed particularly nitrogen (N) and carbon (C); these factors provide a good insight into the dynamics of fynbos riparian zones and the possible alteration because of the invasion of *A. mearnsii*. Additionally, the species can alter decomposition rates instream and to an extent macroinvertebrate assemblages, which might cause the modification and the loss of aquatic processes and species diversity. The work presented here is a holistic approach to understand the nutrient economy of *A. mearnsii* and the effect the species has on macroinvertebrate diversity in the nutrient poor fynbos riparian zones, South Africa.

Overall, *A. mearnsii* had seven to ten times more leaf litterfall in the Wit and Du Toit's River compared to co-occuring native species, with two peaks in litterfall, one at the end of the dry season in mid-autumn; this is a novel finding. The results add to the previous knowledge of terrestrial and riparian biochemistry affected by the inputs under stands of *Acacia* spp., (Milton, 1981; Witkowski, 1991a; Yelenik et al., 2004, 2007; Naude, 2012). *Acacia mearnsii* also keeps a relatively high leaf litter N concentrations year round and decomposes much faster than the less nutrient rich fynbos species. The finding of the high decomposition rates of *A. mearnsii* in streams is another novel find, which is often assumed (Ehrenfeld, 2003; Allison and Vitousek, 2004); however, here it was shown empirically. Additionally, in the study, it was found that

although *A. mearnsii* appears to conserve especially P tighly in their senesced leaves, N is a major component of litterfall in invasive stands, and amount to five to six times more N that is added to riparian soils compared to native species. These interacting pathways from *A. mearnsii* can lead detrimental ecosystem degration in the long term. It also further supports the classification of *A. mearnsii* as a transformer species.

Wiener, (2018) in his study postulated that the high N concentrations in sediments load of the Wit River were due to N rich leaf litter entering the stream from the adjacent riparian zone. The discharge regime in Mediterranean streams of the Fynbos biome is unpredicatable and is season dependent (Gasith and Resh, 1999). Streams in the ecosystems of the biome generally receive litter inputs from native vegetation in December, which is apparent in our study. However, the pulse of nutrient rich litter entering the stream in autumn could have a negative effect, as the system may not be adapted to a large flush of N-rich litter during the transitional phase into winter. It is predicted that rivers systems in parts of South Africa, particularly in the Western Cape, will lose significant stream flow as there are fewer wet years now than 30 years ago (de Moor and Day, 2013). Therefore, it can be significantly detrimental to ecosystems process of these rivers as the leaf litter inputs and the faster decomposing of invasive plants can potentially be stored in stream sediment and act a possible large stock of N. In the future riverine systems will have an inability to flush out excessive sediments that are nutriet enriched due to reduction in stream flow. Consequently, it can lead to a feedback loop in the eutrophication of stream in the Fynbos biome and it may affect in stream aquatic environments notably macroinvertebrates.

de Moor and Day, (2013) noted that macroinvertebrate species in the Fynbos biome are more rich and diverse than the well-documented riparian plant communities; however, knowledge around these communities' stil remains sparse. In the present study a large number of individuals, over several genera were sampled, with deposit feeders, scrapers and predators the most well represented. As riverine systems dry up, the eutrophication from streams through invasive plants species (e.g. *A. mearnsii*) could lead to the loss of key macroinvertebrate groups, which regulate function in stream ecosystems. However, potential changes were not clear from the results found. It is important to note that riparian zones are dynamic systems, which are complex and connected, and a change in one component may trigger a chain reaction in another (in stream, Terai, 2012). It is clear that invasive *A. mearnsii* possess traits such as high N levels in litter, fast decomposition rates and generally impact the ecosystem with a much larger footprint compared to native species, which can disrupt the balance within the native plant community (Morris et al., 2011). The multiple attributes of *A. mearnsii* allow a self-facilitating effect, which can accelerate further growth and the spread of the species in natural landscapes and alter instream sediment dynamix and potentially eutrophy streams in a bottom-up sequence.

In conclusion, it is imperative that these invasive plants species are cleared along watercourses in South Africa, particularly in the Western Cape, where drought is a major environmental catastrophe. Therefore, restoration project like the Working for Water programme should employ qualified ecologists as the threat of invasive species effect multiple services in an ecosystem, especially given the dynamic nature of riparian and adjacent stream ecosystems.

5.2 Future research

Further research needs to look into the annual trends of phosphorous in leaf litter as high amounts of P (4-8 times higher than natives) was found in senesced leaf litter, despite high retranslocation in the invasive leaves. This suggests the invasion *Acacia* spp., notably *A. mearnsii* is changing fynbos riparian ecosystems through impacts on P cycling (see also Naude, 2012). In the same study areas of the Wit River, Wiener, (2018) studied the impacts of sites invaded by N_2-fixing species (mostly, *A. mearnsii*) on in stream biogeochemical processes and found elevated N and P concentrations in sediment. The results of Naude, (2012) suggest that this is not suprising as in her study high phosphatase activities was found under *A. mearnsii* stands, which can enhance and facilitate further invasion even in undisturbed environments where P availibilty might not be as high. Therefore, the elevated nutrients, notably P found in Wiener, (2018) could have been exported from the riparian area, which led to enhanced P concentrations in sediment particularly at the Wit River, invaded reach. The ability to conserve P tighly is one of the major contributing factors for their success in fynbos ecosystems, particularly in riparian zones. However, the results found might be an underestimation of the resorption potential of P in the species as resorption was only determined in autumn, therefore, resorption potential needs to be determined in other seasons. Van der Colff et al., (2017) suggested that regardless of nutrient availability in the environment *A. mearnsii* would still have the resorption potential, which may be the competitive advantage they have over native species in nutrient limited systems. This warrants a more comprehensive investigation on resorption potential of the species spatially.

Many authors in different landscapes in South Africa (Tye and Drake, 2012; Van der Colff et al., 2017) found that *A. mearnsii* fixed a substantial amount of nitrogen from atmphosheric sources. Further, research needs look into the seasonal biological N_2-fixation of *A. mearnsii* as we did not measure the NDFA%. In addition, future research as well need examine the critical value in soil N where *A. mearnsii* could be shifting from BNF to uptake of soil N. There is some evidence in Australia (Pfautsch et al., 2008) to address this theory but remains unknown in *Acacia* spp. in general.

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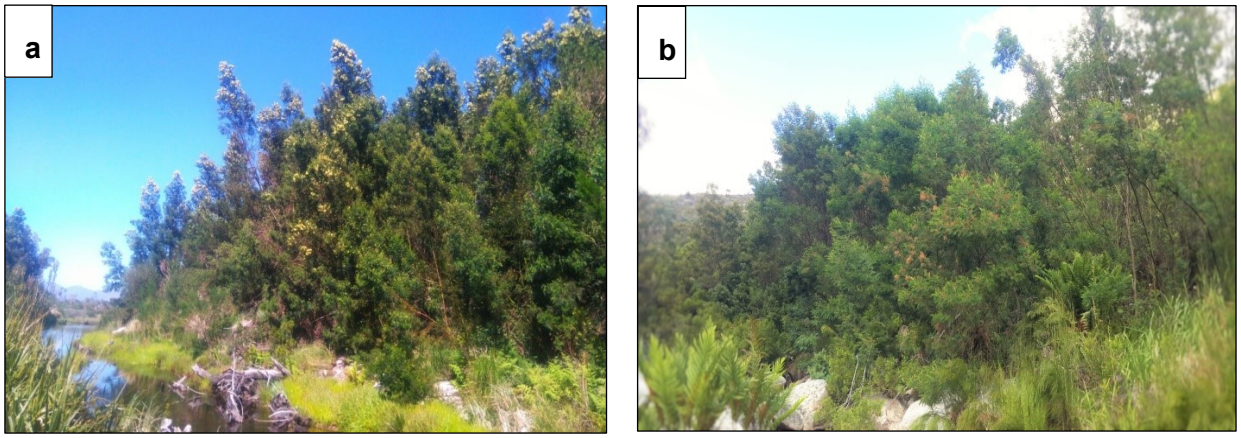
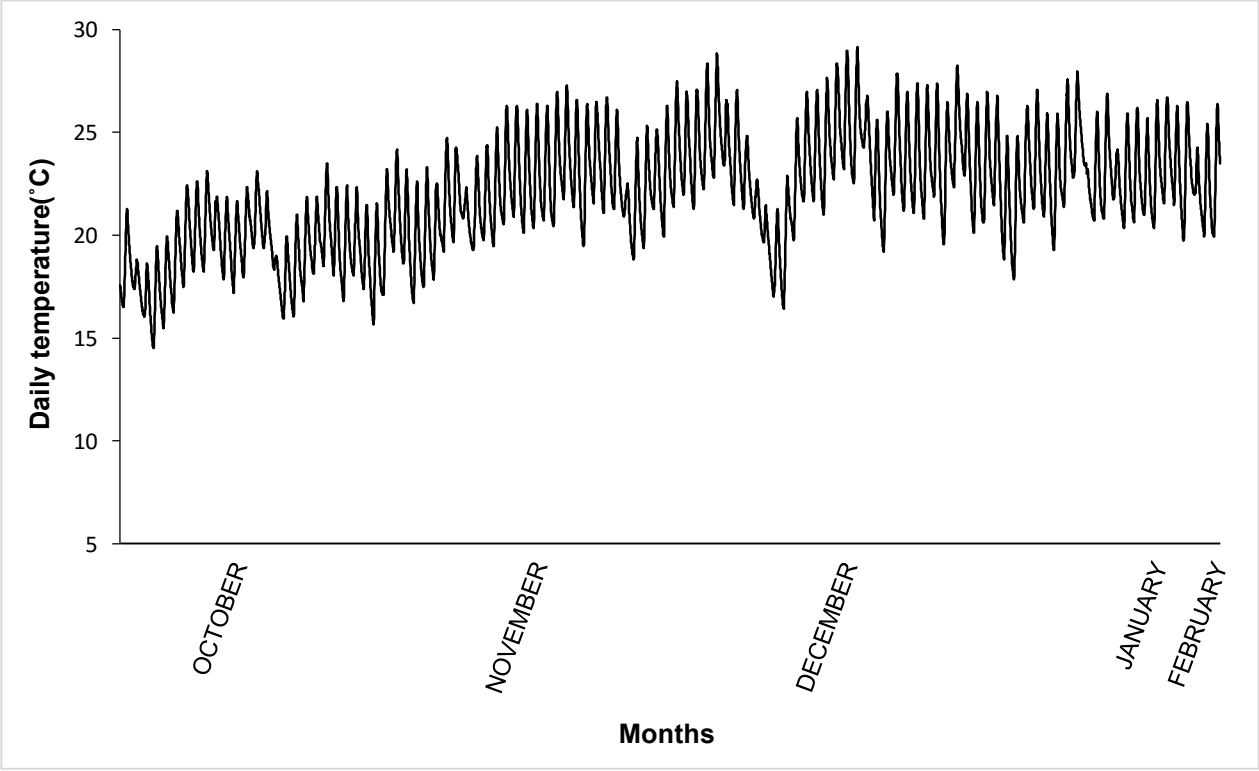
APPENDICES

Appendix A: Representation of flowering season (early) at the (**a**) Du Toit's River, invaded site on the 20th October 2016 showing pale yellow flowers and late flowering season at the (**b**) Wit River invaded site on the 11th November 2016 showing orange flowers.

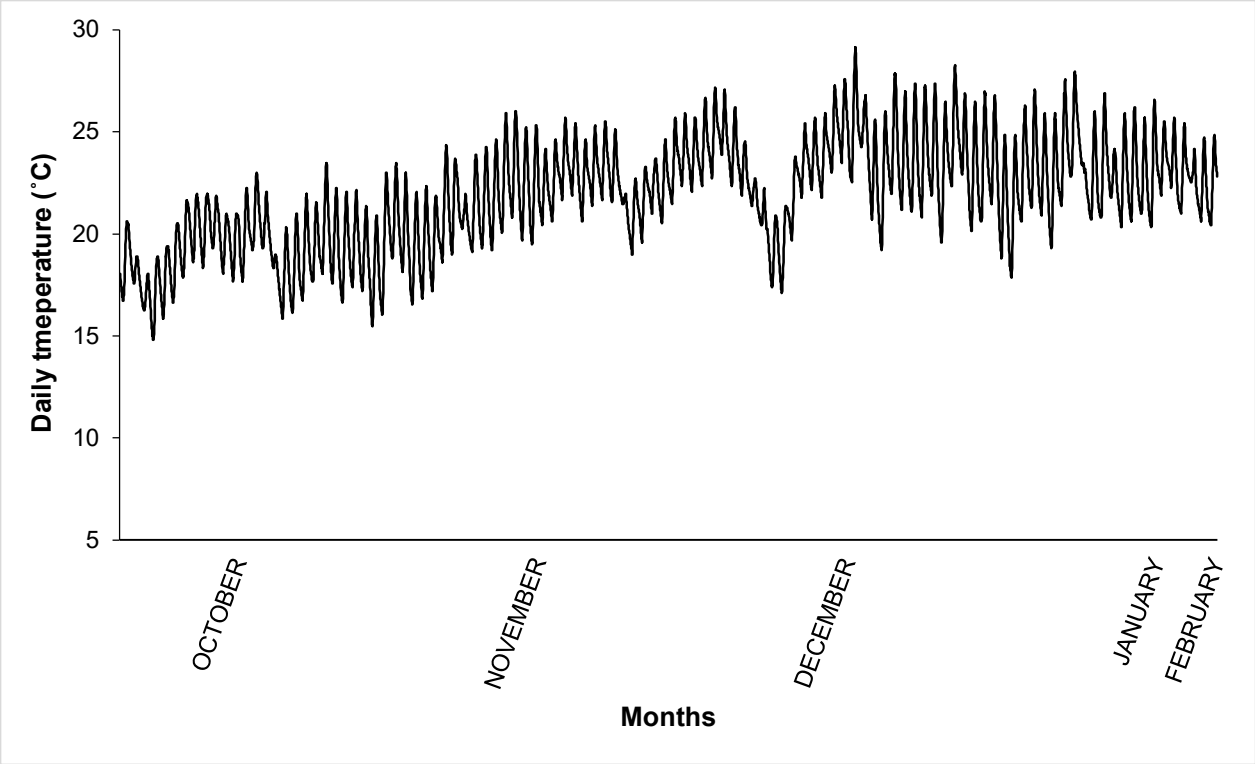


Appendix B: Represent the daily temperature (˚C) at the Wit River, Bainskloof Pass for near pristine (a) and invaded sites (b). Measurements were made from the 4th November 2016 until the 6th February 2017.

(a)

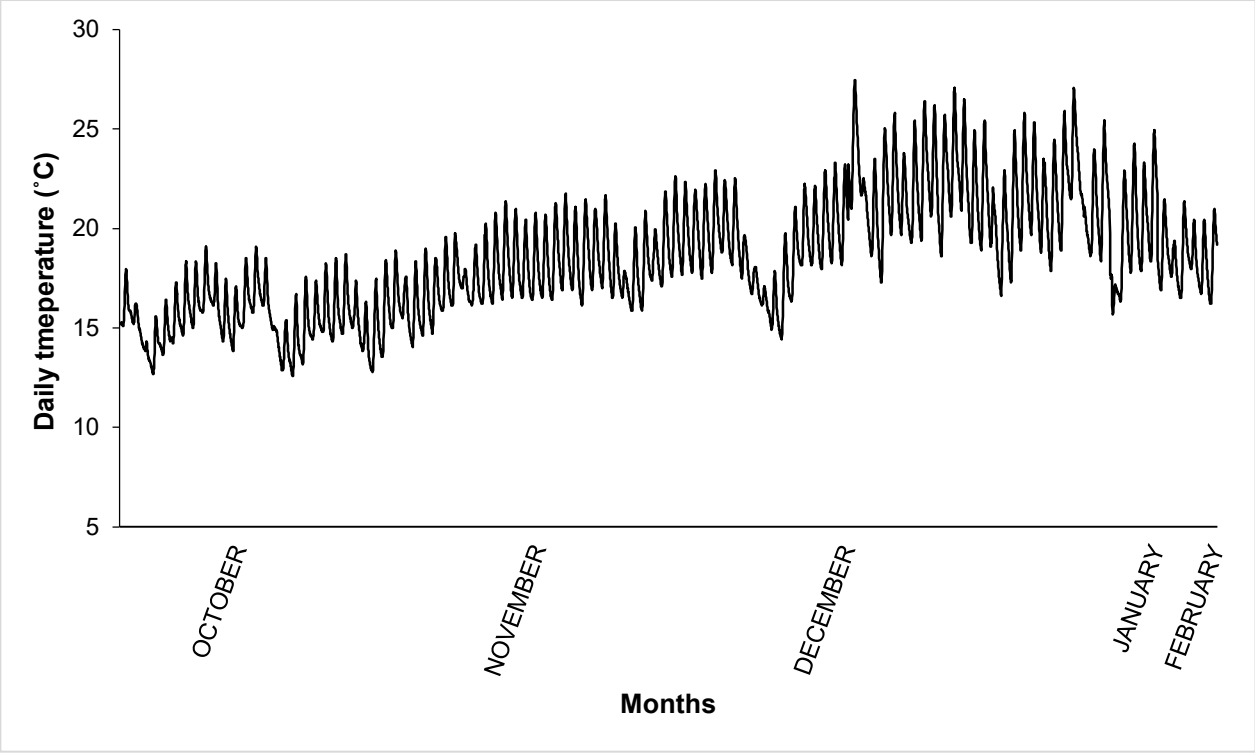


(b)

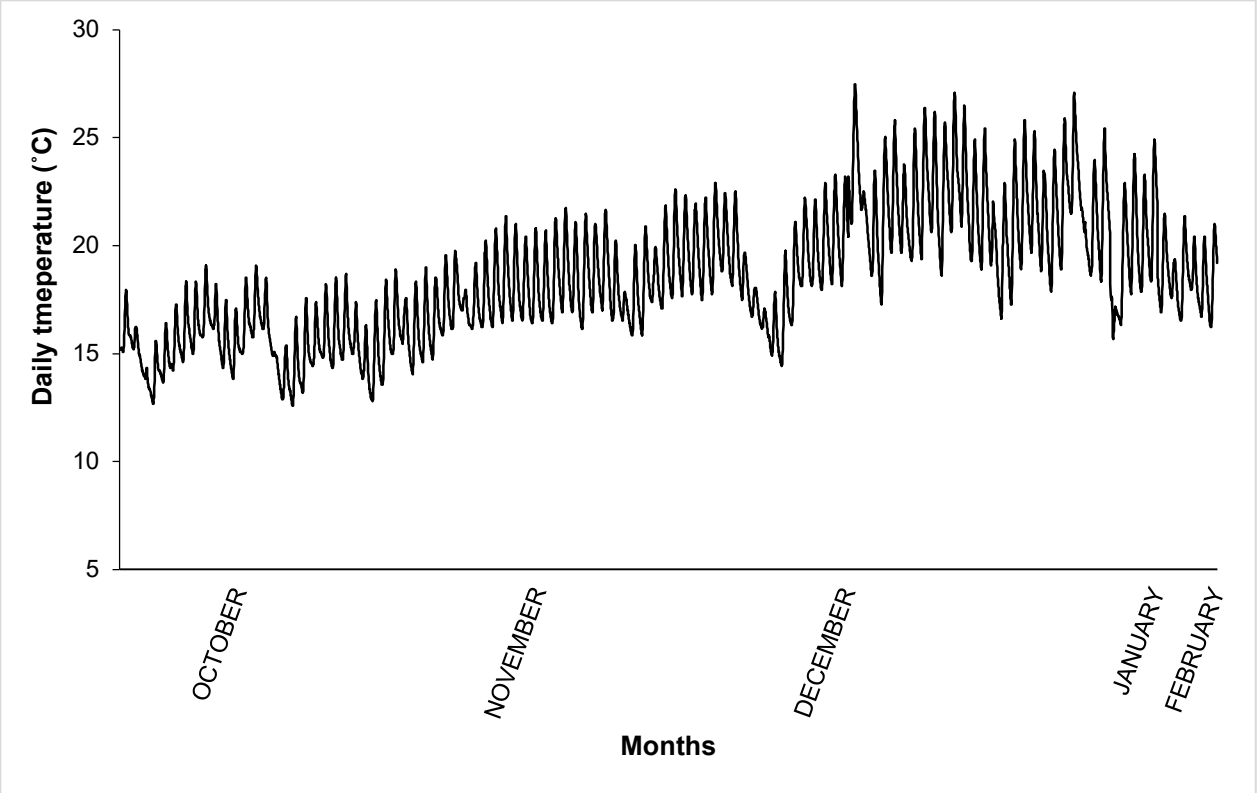


Appendix C: Represent the daily temperature (˚C) at the Du Toit's River, Franschhoek Pass for near pristine (a) and invaded sites (b). Measurements were made from the 4th November 2016 until the 6th February 2017.

(a)



(b)



Appendix D: Table comprises of measurements taken of discharge (m^3 s^{-1}), pH and EC at each incubation day of leaf litter bag collections from the 4th November 2016 until 6th February 2017 at the Wit River (a) and Du Toit's River (b) at different sites.

Site: Wit River (A)
Treatment: Near pristine (a)

Incubation days:	**0**	**14**	**28**	**48**	**64**	**80**	**102**
Discharge (m^3 s^{-1})	0.79	0.78	0.77	0.77	0.81	0.76	0.76
pH	4.62	4.69	4.42	4.74	4.6	4.73	4.47
EC (conductivity)	75.10	75.20	75.10	74.90	74.80	74.90	75.20
Treatment: Invaded site (b)							
Discharge (m^3 s^{-1})	0.77	0.70	0.67	0.67	0.67	0.66	0.66
pH	4.59	4.42	4.41	4.80	4.60	5.07	4.33
EC (conductivity)	75.00	74.60	74.40	74.60	74.80	74.70	74.40

Site: Du Toit's River (B)
Treatment: Near pristine (a)

Incubation days:	0	14	28	48	64	80	102
Discharge (m^3 s^{-1})	0.40	0.30	0.21	0.17	0.17	0.18	0.16
pH	4.85	4.76	4.88	4.92	5.08	4.96	5.12
EC (conductivity)	74.60	74.20	69.70	74.40	74.60	67.30	73.50
Treatment: Invaded site (b)							
Discharge (m^3 s^{-1})	0.71	0.59	0.51	0.47	0.44	0.44	0.43
pH	4.56	4.50	4.69	5.10	4.82	4.68	4.54
EC (conductivity)	74.20	74.10	73.10	74.40	73.40	70.20	74.10

Appendix E: Richness abundance (taxa and functional feeding groups) of macroinvertebrate assemblage species during the sampling events in near pristine and invaded reaches at the Wit River, Bainskloof Pass. The data present different litter types (FS = Fynbos species; AM = *A. mearnsii*) over weeks. HFA indicates the home field species.

Wit River: (Weeks/Interval)		2										4														6											
Treatment: near pristine/invaded		Near pristine				invaded						Near pristine							invaded								near pristine						invaded					
Taxa	FFG	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS (HFA)	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS	FS	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS	FS
Adenophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anchytarsus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthripsodes	Deposit Feeder	0	0	0	1	0	1	0	0	0	0	1	4	1	0	1	0	0	2	6	1	1	0	1	0	2	0	1	1	0	1	0	4	2	2	1	6	0	0
Aprionyx	Deposit Feeder	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidytidytes	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Athericidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Barbarochthon	Grazer	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	1	0	0	0	0	0	0	0	0	0	0
Caenis	Deposit Feeder	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanophlebia	Deposit Feeder	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
Ceratopogoninae	Grazer	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorolestes	Predator	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chloroniella peringueyi	Predator	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroterpes	Deposit Feeder	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	1	1	0	0	0	0	2	2	2	2	0	0	0	2	0	1	1	4
Cloeon	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctenelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dolophilodes	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryopidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops lutulentus	Scraper	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ecnomus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ellattoneura	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enochrus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eukiefferiella	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helodidae	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hydrochus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilinae	Predator	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Laccobius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larsia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptecho helicotheca	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Leptocerus	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0	0	1	0	0	0
Leptophlebiae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycosidae	Predator	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nanocladius	Scraper	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notiothemis	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonemouridae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orectogyrus	Predator	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Orthocladiinae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pachyelmis convexa	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramelitidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramerina	Predator	0	0	0	2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Paraphaenocladius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parecnomina	Predator	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllomacromia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prosthetops brincki	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rapmus	Scraper	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Setodes barnardi	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strina aequalis	Scraper	0	0	1	0	0	0	0	0	0	0	6	0	3	0	1	0	0	0	4	0	7	0	0	2	4	1	0	2	1	3	4	1	0	4	1	3	0	0	1
Sympetrum fonscolombii	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchortus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanypodinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thienemaniella	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	2
Richness per sampling week and litter type		2	3	2	4	1	1	1	3	3	3	9	4	5	3	4	3	6	7	10	4	11	4	3	6	6	2	2	7	8	11	8	8	3	7	5	10	3	1	8
Richness per site		11				12						34							51								38						36					

Wit River: (Weeks/Interval)		8																10																12													
Treatment: near pristine/invaded		near pristine								invaded								near pristine								invaded								near pristine								invaded					
Taxa	FFG	FS (HFA)	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS		FS (HFA)	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS	FS	FS (HFA)	FS (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS	FS
Adenophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anchytarsus	Scraper	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0
Anthripsodes	Deposit Feeder	0	0	0	0	0	0	0	3	1	1	2	1	1	2	1		0	3	0	1	7	4	0	5	7	0	4	2	5	5	3	12	3	0	2	3	0	2	6	1	7	16	9	4	4	9	2	3
Aprionyx	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0		0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3	0	0	0	0	4	2	1	1	3	0	5	1
Aspidytidytes	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Baetidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Barbarochthon	Grazer	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0		0	0	1	4	3	2	2	0	0	0	0	0	0	4	1	3	3	1	2	0	3	1	1	1	0	1	0	2	0	0	0	0
Caenis	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1	0	0	1	0	0	4	1	5	2	6	6	1	4	0	0	0	1	0	0	1	0	1	7	1	0	1	3	1	1
Castanophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	1	0	0	0	0	0
Ceratopogoninae	Grazer	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Chlorolestes	Predator	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroniella peringueyi	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroterpes	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0		0	0	0	1	0	0	0	0	2	1	3	4	1	0	1	0	2	3	0	2	8	0	3	3	3	1	1	1	0	0	0	1
Cloeon	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctenelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Dolophilodes	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryopidae	Scraper	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops lutulentus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecnomus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ellattoneura	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Elmidae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enochrus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eukiefferiella	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helodidae	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laccobius	Scraper	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larsia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptecho helicotheca	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0	0	0
Leptelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptocerus	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophledae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycosidae	Predator	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0		0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nanocladius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notiothemis	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonemouridae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orectogyrus	Predator	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orthocladiinae	Scraper	0	0	0	0	0	0	0	1	2	0	0	1	1	2	0		0	0	1	0	0	0	0	0	0	1	2	1	0	0	0	1	0	0	0	0	3	3	0	0	1	3	0	1	3	0	1	2
Pachyelmis convexa	Scraper	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramelitidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramerina	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0
Paraphaenocladius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parecnomina	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllomacromia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prosthetops brincki	Scraper	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rapmus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Setodes barnardi	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strina aequalis	Scraper	5	4	7	9	2	0	2	7	6	2	4	4	8	5	14		2	10	19	12	39	20	18	46	0	12	11	18	5	1	20	18	2	5	6	2	5	11	2	12	5	5	1	6	4	0	8	4
Sympetrum fonscolombii	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchortus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tanypodinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thienemaniella	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Richness per sampling week and litter type		7	6	7	11	2	2	3	16	10	3	7	8	11	10	15		2	14	24	19	49	30	21	51	15	17	28	27	18	16	28	42	17	10	13	14	20	18	15	20	23	35	16	16	17	##	18	12
Richness per site		54								64								210								191								127								149							

Appendix F: Richness abundance (taxa and functional feeding groups) of macroinvertebrate assemblage species during the sampling events in near pristine and invaded reaches at the Du Toit's River, Franschhoek Pass. The data present different litter types (FS = Fynbos species; AM = *A. mearnsii*) over weeks. HFA indicates the home field species.

Du Toit's River: Weeks/Intervals		2							4								6											
Treatment: near pristine/invaded		Near pristine			invaded				Near pristine						invaded		Near pristine				invaded							
Taxa	FFG	FS (HFA)	FS (HFA)	AM	AM (HFA)	AM (HFA)	FS	FS	FS (HFA)	FS (HFA)	AM	AM	A M	A M	AM (HFA)	AM (HFA)	FS (HFA)	FS (HFA)	AM	AM	AM (HFA)	AM (HFA)	AM (HFA)	FS	FS	FS	FS
Adenophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anchytarsus	Scraper	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthripsodes	Deposit Feeder	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1
Aprionyx	Deposit Feeder	1	1	1	0	0	0	1	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Aspidytidytes	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Barbarochthon	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenis	Deposit Feeder	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Castanophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogoninae	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Chlorolestes	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroniella peringueyi	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroterpes	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cloeon	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctenelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dolophilodes	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryopidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops lutulentus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecnomus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1	0	0	0	0	0
Ellattoneura	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enochrus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eukiefferiella	Deposit Feeder	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helodidae	Grazer	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Laccobius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larsia	Predator	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptecho helicotheca	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptocerus	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycosidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nanocladius	Scraper	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notiothemis	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonemouridae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orectogyrus	Predator	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Orthocladiinae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pachyelmis convexa	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramelitidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramerina	Predator	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0
Paraphaenocladius	Scraper	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Parecnomina	Predator	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllomacromia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prosthetops brincki	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rapmus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Setodes barnardi	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Strina aequalis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0
Sympetrum fonscolombii	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchortus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanypodinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thienemaniella	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	1	0	2	2	2
Richness per sampling week and litter type		1	2	3	2	3	1	1	2	2	2	1	1	1	2	4	5	1	5	1	7	1	5	1	3	3	4
Richness per site		13							9						6		12				24						

Du Toit's River: Weeks/Intervals		8							10							12								
Treatment: near pristine/invaded		Near pristine				invaded			Near pristine		invaded					Near pristine			invaded					
Taxa	FFG	FS (HFA)	FS (HFA)	FS (HFA)	FS (HFA)	AM (HFA)	AM (HFA)	AM (HFA)	FS (HFA)	FS (HFA)	AM (HFA)	FS	FS	FS	FS	FS (HFA)	AM	AM	AM (HFA)	AM (HFA)	FS	FS	FS	FS
Adenophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anchytarsus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Anthripsodes	Deposit Feeder	0	0	0	0	0	0	0	0	0	2	1	0	2	0	0	0	1	1	0	1	0	0	3
Aprionyx	Deposit Feeder	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspidytidytes	Predator	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
Baetidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Barbarochthon	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenis	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	9	20	10	0	1	9	13	19
Castanophlebia	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogoninae	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Chlorolestes	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroniella peringueyi	Predator	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroterpes	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cloeon	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ctenelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dolophilodes	Filter Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryopidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops lutulentus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecnomus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Ellattoneura	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enochrus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eukiefferiella	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helodidae	Grazer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laccobius	Scraper	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larsia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptecho helicotheca	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptelmis	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptocerus	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycosidae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nanocladius	Scraper	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notiothemis	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonemouridae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orectogyrus	Predator	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Orthocladiinae	Scraper	0	0	0	0	0	0	0	0	0	1	1	2	1	3	1	2	5	3	1	1	0	1	1
Pachyelmis convexa	Scraper	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramelitidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramerina	Predator	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2	0	0	0
Paraphaenocladius	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parecnomina	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllomacromia	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prosthetops brincki	Scraper	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rapmus	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Shredder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Setodes barnardi	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	Filter Feeder	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strina aequalis	Scraper	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Sympetrum fonscolombii	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Synchortus	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanypodinae	Predator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thienemaniella	Scraper	0	5	0	0	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Richness per sampling week and litter type		3	9	1	1	6	2	3	1	3	3	2	2	4	6	2	11	28	14	2	8	10	16	23
Richness per site		14				11			4		17					41			73					